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CLIMATIC SIGNIFICANCE OF HYDROGEN AND OXYGEN ISOTOPIC RATIOS IN TREE RINGS

by

© Se Jong Song

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
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OF DOCTOR OF PHILOSOPHY IN PHYSICS (GEOPHYSICS)

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled Climatic Significance of Hydrogen and Oxygen Isotopic Ratios in Tree Rings submitted by Se Jong Song in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Geophysics.

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ABSTRACT

This study examines the possible use of hydrogen and oxygen isotope records preserved in tree rings as paleotemperature indicators. The study consists of two parts. The first part deals with the methodology whereby the isotopic composition of tree rings can be determined. The second part investigates the significance of the isotope records as a paleo-thermometer.

A new method of measuring both D/H and $^{18}O/^{16}$ ratios in organic compounds is discussed. This technique involves the pyrolysis of organic compounds in a nickel reaction vessel and utilizes diffusion of hydrogen gas through the thin walls of a nickel reaction vessel. This method enables quantitative extraction of hydrogen and oxygen from tree rings for isotopic analysis by mass spectrometry. The method gives reproducible and accurate results with a variety of organic compounds and standard waters.

In order to eliminate any fractionation problems associated with chemical heterogeneity in tree rings, cellulose was chosen as the working material. For hydrogen isotopic analysis, the cellulose was nitrated to eliminate the exchangeable OH hydrogen in the cellulose. Two methods of nitration were tested. One method (W-C-CN) involves the extraction of α-cellulose from wood mill followed by nitration. The other method (W-CN) involves direct nitration of the wood mill. The first method (W-C-CN) appears preferable to the latter (W-CN) in terms of reproducibility and correlation with climatic parameters.

The D/H ratios of cellulose nitrate extracted from the wood of three spruce trees, which grew in Edmonton, were measured. All three trees showed a sudden shift in D/H ratios between juvenile and mature sections of the tree. In each case the juvenile sections are depleted in deuterium compared to the mature sections. The phenomenon is not present in the oxygen isotope records, nor is it due to climatic changes. Possible explanations for this phenomenon are given.

Both D/H and $^{18}O/^{16}O$ ratios from cellulose in tree rings show functional relationships with climatic parameters. D/H ratios in cellulose nitrate show a strong linear correlation with temperature at the growth site. $^{18}O/^{16}O$ ratios in cellulose are less well correlated with temperature but are still significant.

Strong positive linear correlations between isotopic compositions and winter temperature, together with negative correlations with the amount of precipitation, indicate that the isotopic composition of soil water is the primary factor controlling isotopic compositions of tree rings.

Both hydrogen and oxygen isotope models are developed. The hydrogen isotope model incorporates the evapotranspiration model of Dongmann et al (20) in which no temperature-dependent fractionation is assumed. The oxygen model assumes that oxygen in cellulose is derived from atmospheric CO_2 which is in complete equilibrium with leaf water and contains a temperature-dependent fractionation term between CO_2 and water. The models developed here are in excellent agreement with the experimental results obtained with tree ring samples from various localities of North America.

The study shows that while the isotopic enrichment of cellulose with respect to soil water is a function of relative humidity, paleo-humidity information cannot be obtained from isotope data because paleo-isotopic compositions of waters are unknown. The best climatic information from isotopic records in tree rings appears to be temperature variation, particularly that of mean annual temperature, which does not require a priori knowledge of isotopic composition of soil water.

In summary, this study shows that isotopic compositions, particularly those of hydrogen, preserved in tree rings, provide a measurement of paleo-temperature changes occurring during the life span of the tree.

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CHAPTER I

INTRODUCTION

It is generally accepted that global climate has undergone considerable variation in the past. It is to be expected that such variation will continue in the future. Considering the vital impact of climate on human life and the uncertainties associated with future climatic variations, the development of an ability to forecast these future variations is an important and challenging task. In addition to natural variations which have been observed, there is a growing belief that human activities, especially recent industrial developments may have considerable influence on future climate (75).

Study of the past climate variation is essential for the development of climate models which may form the basis for prediction of future climate trends. Over the past few centuries, the development of meteorological instruments has enabled the collection of data which has allowed quantitative analysis of climate and climate variations. The study of climatic variations, however, cannot depend entirely on instrumental data which extend back only a relatively short time period. Additional climatic information must come from proxy records, such as fossil pollen, mountain glaciers, sediments from lakes and oceans, and isotope data.

As early as 1948, Urey (99) proposed that paleotemperatures of the ancient oceans could be estimated by measuring oxygen isotope distribution between calcium carbonate and water. The method is based on the observation that the oxygen isotopic composition of $CaCO_3$ differs from that of water at equilibrium and that the difference is temperature-dependent. Since Urey's proposal many researchers throughout the world have used this principle to investigate paleoclimate variations. The study of paleotemperatures in the Pleistocene was pioneered by Emiliani (23,24,25). By measuring the oxygen isotopic composition of planktonic foraminifera from deep-sea sediments, Emiliani assembled a detailed record of temperature variations during the Pleistocene epoch. However, Shackleton et al (89) demonstrated that the observed isotope variations reflect primarily the changing volume of polar ice. The generalized paleotemperature curve thus revised by Emiliani and Shackleton (26) revealed recurring temperature fluctuations extending for about 700,000 years into the past.

Another successful application of isotope techniques to paleotemperature reconstruction has been reported by Dansgaard et al (17) and Lorius et al (71). They showed that the oxygen isotopic composition in each accumulating layer of polar ice caps is closely related to the temperature at the site of precipitation. Isotope records from the ice caps are in excellent agreement with those of calcium carbonate from deep-sea sediments for the equivalent periods. Thus, they yield temperature information tracing back a few hundred thousand years.

Bio-organic materials that are deposited year after year have been reported to preserve climatic information in the form of stable isotope abundances (65). In general, D/H and $^{18}O/^{16}O$ ratios in modern meteoric water depend upon the temperature at the site of precipitation (16). Thus the isotopic composition of biological systems utilizing meteoric water may preserve the record of past temperature variations. In addition, if the photosynthetic process leads to temperature-dependent isotopic fractionation between different photosynthates in the biological system, a detailed record of temperature variations covering several thousand years may be obtained. In particular, tree rings have great potential as climatic indicators for a number of reasons. Dendrochronology provides an absolute dating mechanism and relatively long continuous climatic records are possible.

Like any man-made sensing device, natural paleoclimatic indicators must be calibrated and their distinctive performance characteristics must be understood before rigorous paleoclimatic reconstruction is possible. Therefore, the main objective of this work is to investigate the existence of possible isotope thermometers in tree ring sequences, their significance and calibration.

CHAPTER II

BACKGROUND

1. Isotopes and their Properties

It is now known that almost all the elements have more than one naturally occurring isotope. Isotopes of an element have the same number of protons but slightly different atomic masses depending upon the varying number of neutrons in their nuclei. As a result, their chemical and physical properties are not identical.

The chemical properties of an element are largely determined by the number and arrangement of their orbital electrons. Thus, the isotopes of an element, having the same number of electrons arranged in a similar manner, would have similar chemical properties. Research done since 1930, however, has revealed that the isotopes of an element and their compounds differ slightly in their chemical properties and that the differences in chemical properties are due to the differences in their thermodynamic properties associated with their masses. It was also recognized that the isotopes differ in their physical properties that are directly related to mass, such as diffusion rate, rate of evaporation, vapor pressure, and density of gaseous and condensed phases.

During the early stage of isotope studies it was believed that the stable isotopes of elements occur in nature in constant proportions. With the introduction of mass spectrometric methods for the determination of the abundance of the stable isotopes, it became evident that the isotopic composition of elements, especially those of low atomic numbers are variable because their isotopes are fractionated in the course of certain chemical and physical processes occurring in nature. This fractionation is proportional to the difference in masses between two isotopes. The higher the mass difference between two isotopes, the greater the fractionation effect. For this reason stable isotope studies have tended to concentrate on commonly occurring low atomic mass elements such as hydrogen (D/H), carbon ($^{13}C/^{12}C$), nitrogen ($^{15}N/^{14}N$), oxygen ($^{18}O/^{16}O$) and sulphur ($^{34}S/^{32}S$).

2. Natural Isotope Variations

In general, isotopic fractionation occurs during several kinds of chemical and physical processes (79,32).

1. Isotopic exchange reactions involving the redistribution of isotopes of an element among different molecules containing that element. For example,

   $$\frac{1}{2}\ Si^{16}O + H_2{}^{18}O \rightleftharpoons \frac{1}{2}\ Si^{18}O_2 + H_2{}^{16}O.$$

2. Unidirectional reactions in which the difference in reaction rate between two isotopic atoms or molecules leads to fractionation. The differences in reaction rates are due to differences in thermodynamic properties, such as energy, entropy and the free

energy of the species.

3. Physical processes such as evaporation and condensation, melting and crystallization, adsorption and desorption in which mass differences come into play.

3. Equilibrium Constant in Isotope Exchange Reactions

The thermodynamic properties of isotopic molecules and the resulting fractionation of isotopes have been studied by a number of researchers (5,99). Quantum mechanics shows that the energy levels of molecules are mass dependent. Thus isotopic species have different energy levels which result in a difference in the behaviour of the isotopes of an element in certain chemical reactions. The fractionation that occurs in such natural processes is indicated by a fractionation factor which is defined as

$$\alpha_{AB} = R_A/R_B \qquad [1]$$

where R_A is the isotopic ratio such as D/H or $^{18}O/^{16}O$ in molecule A or phase A and R_B is the same in phase B.

A typical isotope exchange reaction between two molecules A and B may be written as

$$A_1 + B_2 \rightleftarrows A_2 + B_1 \qquad [2]$$

where subscripts 1 and 2 represent the light and the heavy isotopes respectively. Then the equilibrium constant for this reaction is given in terms of partition functions Q by

$$K = \left(\frac{Q_2}{Q_1}\right)_A \Bigg/ \left(\frac{Q_2}{Q_1}\right)_B . \qquad [3]$$

At equilibrium, the fractionation factor α is equal to the equilibrium constant k:

$$\alpha_{AB} \cong K. \qquad [4]$$

If the fractionation factor is greater than unity, the products of reaction [2] will be favoured, while if it is less than unity, the reactants will dominate. Equation [3] indicates that the equilibrium constant or fractionation factor is determined only by the ratios of partition functions. The partition function is defined as

$$Q = \sum_i e^{-E_i/kT} \qquad [5]$$

where k is the Boltzman constant, T is the absolute temperature and E_i is the energy at quantum state i (3). Equations [3] and [5] indicate that fractionation is a function of the energy of the molecule and the temperature of the system.

The energy of a molecule in a gas can be described in terms of the electronic interactions plus translational, rotational and vibrational components of the atoms in that molecule. Thus the overall energy of the system can be written as

$$E = E_{elect.} + E_{trans.} + E_{rot.} + E_{vib.} \qquad [6]$$

Accordingly, the overall partition function of the molecule becomes the product of individual partition functions associated with the corresponding energies.

$$Q = (Q_{elect.}) \times (Q_{trans.}) \times (Q_{rot.}) \times (Q_{vib.}). \qquad [7]$$

In general, the partition function due to electronic energy does not contribute to isotope fractionation except in the case of hydrogen. The excited electronic levels of the atoms or molecules of interest lie so far above the ground state that only at temperatures of several thousand degrees does the term kT become comparable with the energy term E_i and the partition function assumes significant probability. Thus only the ground electronic state of degeneracy g_o contributes to the energy and the electronic partition function becomes simply the degeneracy

$$Q = g_o.$$

When only the ratio of partition functions is considered, it becomes unity so that no isotopic fractionation occurs.

The translational partition function is given by

$$Q_{trans.} = \left(\sum_{n=1}^{\infty} e^{-n^2 \; {}^2h^2/2ma^2kT} \right)^3 \qquad [8]$$

where m = atomic mass, a = length of motion, a^3 = volume of gas, n = quantum number, and h = planck constant. Replacing the sum by the integral leads to virtually no loss of accuracy and the translational partition function becomes

$$Q_{\text{trans.}} = \left(\int_0^\infty dn\ e^{-n^2\pi^2h^2/2ma^2kT} \right)^3$$

$$= V\left(\frac{MkT}{2\pi h^2}\right)^{3/2} \qquad [9]$$

where M = molecular mass, V = volume of gas.

The rotational partition function is given by

$$Q_{\text{rot.}} = \frac{1}{\sigma} \sum_{j=0}^{\infty} (2j+1)e^{-j(j+1)h^2/2IkT} \qquad [10]$$

where σ = symmetry number is 1 for an asymmetric molecule and 2 for a symmetric linear molecule, I = moment of inertia and j = rotational quantum number. For $T >> \frac{h^2}{2Ik}$, the equation reduces to a simpler form by making a change of variable $z = j(j+1)$ and $dz = (2j+1)dj$:

$$Q_{\text{rot.}} = \frac{1}{\sigma} \int_0^\infty dz\ e^{-zh^2/2IkT} = \frac{2IkT}{\sigma h^2}\,. \qquad [11]$$

The form of the vibrational partition function neglecting anharmonicity is

$$Q_{\text{vib.}} = \pi_i \sum_V e^{-(V+\frac{1}{2})h\nu_i/kT} = \pi_i \frac{e^{-U_i/2}}{1-e^{-U_i}} \qquad [12]$$

where $U_i = h\nu_i/kT$, ν_i = vibrational frequency, V = vibrational quantum number.

Thus the overall partition function ratio between the light and heavy components of a molecule is given by

$$\frac{Q_2}{Q_1} = \frac{\sigma_1 I_2}{\sigma_2 I_1}\left(\frac{M_2}{M_1}\right)^{3/2} \pi_i \left(\frac{e^{-U_{2i}/2}}{1-e^{-U_{1i}}}\right) \Bigg/ \left(\frac{e^{-U_{1i}/2}}{1-e^{-U_{1i}}}\right). \qquad [13]$$

The translational partition function at all temperatures is classical as is the rotational partition function at normal temperatures except for some molecules containing hydrogen. According to a theorem of Teller and Redlich (99)

$$\frac{I_2}{I_1}\left(\frac{M_2}{M_1}\right)^{3/2}\left(\frac{m_1}{m_2}\right)^{3/2} \pi_i \frac{U_{1i}}{U_{2i}} = 1. \qquad [14]$$

Then the overall partition function becomes

$$\frac{Q_2}{Q_1} = \frac{\sigma_1}{\sigma_2}\left(\frac{m_2}{m_1}\right)^{3/2} \pi_i \frac{U_{2i}}{U_{1i}} \left(\frac{e^{-U_{2i}/2}}{1-e^{-U_{2i}}}\right) \Bigg/ \left(\frac{e^{-U_{1i}/2}}{1-e^{-U_{1i}}}\right). \qquad [15]$$

The ratio of the masses of the isotopes cancels out in any chemical reaction (5). The ratio of the symmetry numbers, regardless of its value, will not lead to isotopic fractionation since it merely represents the relative probabilities of forming symmetrical and unsymmetrical molecules. Thus the classical ratio $(m_2/m_1)^{3/2}$ is omitted in calculation of the equilibrium constant. This omission leads to the equilibrium constant being the ratio of the equilibrium constants for the dissociation of the two isotopic molecules into atoms. A new partition function ratio is defined as

$$\frac{Q_2'}{Q_1'} = \frac{Q_2}{Q_1} \cdot \frac{\sigma_2}{\sigma_1}\left(\frac{m_1}{m_2}\right)^{3/2} = \pi_i \frac{U_{2i}}{U_{1i}} \cdot \frac{e^{-U_{2i}/2}}{e^{-U_{1i}/2}} \, \frac{1-e^{-U_{1i}}}{1-e^{-U_{2i}}}. \qquad [16]$$

By putting $U_{1i} = U_{2i} = \Delta U_i$ and $U_{2i} = U_i$, the equation can be rewritten as

$$\frac{Q'_2}{Q'_1} = \pi_i \frac{U_i}{U_i+\Delta U_i} e^{\Delta U_i/2} \frac{1-e^{-(U_i+\Delta U_i)}}{1-e^{-U_i}} . \qquad [17]$$

Taking the natural logarithm of both sides of equation [17] and utilizing series expansion yields:

$$\ln(Q'_2/Q'_1) = \sum_i \left(\frac{1}{2} - \frac{1}{U_i} + \frac{1}{e^{U_i}-1} \right) \Delta U_i = \sum_i G\Delta U_i \qquad [18]$$

where $G = \frac{1}{2} - \frac{1}{U_i} + \frac{1}{e^{U_i}-1}$.

Thus the final form of the fractionation factor is given by

$$\ln \alpha_{AB} = \left(\sum_i G\Delta U_i \right)_A - \left(\sum_i G\Delta U_i \right)_B . \qquad [19]$$

This equation indicates that the fractionation factor is a function only of the vibrational frequencies of the molecules involved and the temperature of the system. However, in an isotopic exchange reaction, the vibrational frequencies of the molecules involved are fixed. Thus the temperature of the system becomes the only variable affecting isotopic fractionation between the coexisting molecules in the reaction. This is the basic principle underlying the isotope thermometry.

A plot of G vs U_i in Fig. 1 (5) shows that for high frequencies and low temperature ($U_i > 20$), the function G approaches the value 1/2 and ln Q_2/Q_1 is proportional to 1/T. Consequently, $\ln\alpha_{AB}$ is a function of 1/T. For low frequencies and high temperature ($U_i < 3$), the function G has a slope 1/12 and $\ln\alpha_{AB}$ is proportional to $1/T^2$. At even higher temperature the fractionation factor becomes unity and no isotope fraction occurs.

The theoretical considerations presented here were developed for ideal gases and extension of them to the condensed phase is complicated by additional factors such as lattice vibrational frequencies and interactions between the lattice and the internal vibrations. However, this theory serves as an excellent guide and similar temperature dependences of fractionation factors have been observed in both laboratory and natural systems.

4. Chemical Processes

In the previous section, it was shown that vibrational motion of molecules is responsible for isotopic fractionation in chemical reactions. However, the vibrational motion of polyatomic molecules is complex. Since the normal coordinates of a polyatomic molecule resemble independent vibrations, the study of simple harmonic motion of a diatomic molecule provides an adequate background for the understanding of more complex systems. The classical vibrational frequency for the simple harmonic oscillator is

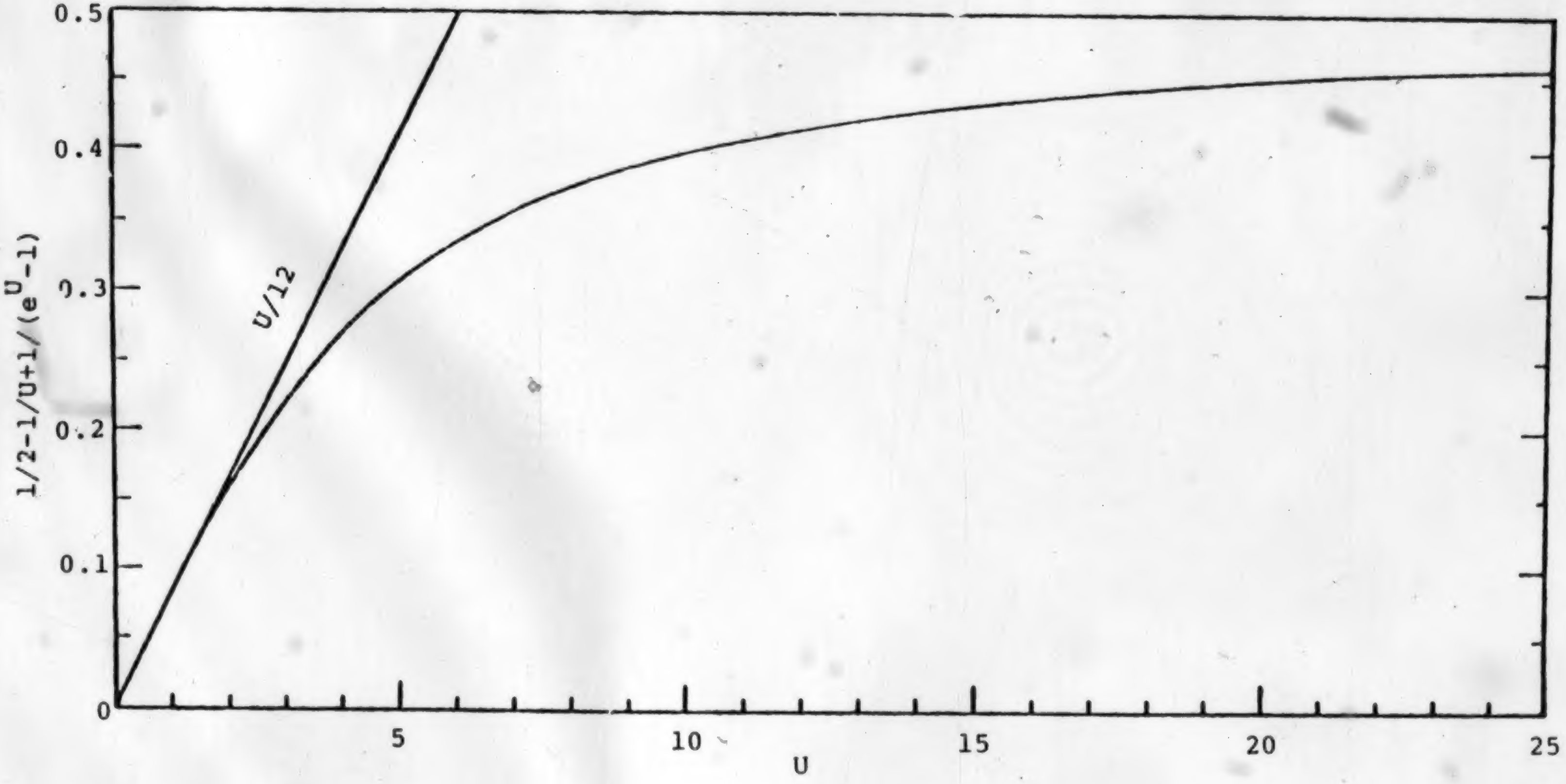


Figure 1. The separate effect in the free energy per unit shift in U, plotted G against U = kcω/kT (5).

$$\nu = \frac{1}{\pi}\sqrt{\frac{K}{\mu}} \qquad [20]$$

where μ = reduced mass $\frac{m_1 m_2}{m_1+m_2}$ and k = force constant. The force constant K is an approximate indication of the strength of the chemical bond (85). Therefore, the isotopic properties of a molecule depend largely upon the nature of the chemical bonds within the molecule and the reduced mass of the vibrating system in the molecule. Thus when isotopic fractionation between the molecules is considered, factors such as oxidation state, ionic charge, atomic mass and electronic configuration of the elements to which the isotope is bonded play an important role (79).

In general, bonds to ions with high ionic potential and low atomic mass are associated with high vibrational frequencies and therefore have a tendency to incorporate the heavy isotope preferentially in order to lower the free energy of the system. For example, in natural equilibrium assemblages, quartz (SiO_2) is always the most ^{18}O-rich mineral and magnetite (Fe_3O_4) is the most ^{18}O-depleted one (11,4). The small highly charged Si^{4+} ion bonded to oxygen has a higher potential energy than does the relatively large Fe^{2+} ion and therefore incorporates ^{18}O preferentially. Taylor (94) found a relatively large ^{18}O difference (1.68°/₀₀) between grossularite ($Ca_3Al_2(SiO_4)_3$) and andradite ($Ca_3Fe_2(SiO_4)_3$) at 600°C. Since both Al and Fe are in the same oxidation state (+3) in grandite garnet, the isotopic difference comes primarily from the mass difference between ^{27}Al and ^{56}Fe.

The isotope fractionation will usually decrease with increasing temperature since the difference in free energy between two isotopic species becomes less significant as the total energy of the system increases. This is illustrated in Table 1 where the fractionation factor for the CO_2-H_2O system is shown for various equilibrium temperatures (6).

Table 1. Variation of $\alpha_{H_2O-CO_2}$ with temperature (6)

α	T °C
1.04606	0
1.04564	2
1.04283	15
1.04075	25
1.03881	35
1.03696	44.7

5. Physical Processes

Physical processes are equally important in producing natural variations in isotope abundances. Among these, evaporation and condensation are the most important processes governing isotopic compositions of precipitation. To understand isotope fractionation occurring in such processes, the meteoric water cycle may be considered. The most abundant isotopic components of water are $H_2{}^{16}O$, $HD^{16}O$ and $H_2{}^{18}O$. Their average relative abundances are approximately 997680:320:2000 ppm respectively (16). The natural abundances of these molecules vary from site to site because evaporation and condensation processes result in isotope fractionation. Fractionation factors for such processes depend upon the temperature and rate of reaction. If the process proceeds slowly, then equilibrium conditions are practically realized at the boundary between the liquid and vapor phases. The fractionation factor between the liquid and vapor phases then becomes simply the ratio of the vapor pressures of the light and heavy components:

$$\alpha = P_1/P_2 \qquad (P_1>P_2) \qquad [21]$$

where subscripts 1 and 2 represent the light and heavy components respectively. At normal temperature, the fractionation factors for HDO and $H_2{}^{18}O$ are approximately 1.08 and 1.009 respectively. Thus when water evaporates, the vapor is depleted in D and ^{18}O. The remaining water which is subject to further evaporation becomes steadily enriched in heavy isotopes D and ^{18}O. As the vapor condenses, the reverse process occurs and HDO and $H_2{}^{18}O$ will tend to concentrate in the condensed phase.

The Clausius-Clapeyron equation predicts that the vapor pressure of isotopic species of water is a function of the molecular mass and the temperature of the system:

$$\ln P = -H/RT + \text{constant} = -mLv/RT + \text{constant} \qquad [22]$$

where m is the molecular mass, Lv is the latent heat of vaporization and H is the enthalpy (85). The effect of temperature on the fractionation factors for water is illustrated in Table 2.

Table 2. Equilibrium fractionation factors for hydrogen and oxygen isotopes at various temperatures (16)

T°C	αD	$\alpha^{18}O$
100	1.029	1.0033
80	1.037	1.0045
60	1.046	1.0058
40	1.060	1.0074
20	1.079	1.0091
0	1.106	1.0111
-10	1.123	1.0123
-20	1.146	1.0135

The αD and $\alpha^{18}O$ values for temperatures t>20°C are those measured by Merlivat et al (74) and Zhavoronkev et al (110). The fractionation factors below 0°C are calculated by extrapolation.

Equilibrium conditions in this system are attained only in practically infinitely slow processes. In reality, non-equilibrium conditions are prevalent and they have important consequences for several steps in evaporation processes in nature. Non-equilibrium processes are often observed in the situation where extremely fast evaporation from a limited water surface occurs as in the cases of closed basins and lakes. The isotopic fractionation in such cases becomes more complicated due to additional kinetic effects introduced during the phase change.

The evaporation from open surfaces has been considered as a molecular diffusion process into the atmosphere (9,73,56). The equation describing the diffusion process is given by

$$j = kD^q \; (C_s - C_a) \qquad [23]$$

where j = water flux, k = wind velocity coefficient, q is a function of the aerodynamical behaviour of the surface, C_s and C_a are saturated and unsaturated molar concentrations of water in the air respectively, and D = molecular diffusivity. Then the kinetic fractionation factor is given by

$$\alpha^k = \left(\frac{D_1}{D_2}\right)^q . \qquad [24]$$

Craig et al (14) have estimated the ratio of diffusion rates from the kinetic theory of gases:

$$\frac{D_2}{D_1} = \left(\frac{M_1}{M_2} \cdot \frac{M_2 + M_G}{M_1 + M_G}\right)^{1/2} \qquad [25]$$

where D_1, D_2, M_1 and M_2 represent the molecular diffusivities and masses light and heavy water and M_G is the molecular mass of the gas into which the water vapor diffuses. Their model values of isotopic fractionations do not account for simultaneously observed D and ^{18}O fractionation. Thus, Merlivat (73) proposed an alternative model in which he assumed a rigid elastic spherical molecule model for water. The ratio of diffusivities from the kinetic theory of gases is given by

$$\frac{D_2}{D_1} = \left(\frac{M_1}{M_2} \cdot \frac{M_2 + M_G}{M_1 + M_G}\right)^{1/2} \left(\frac{\Gamma_1 + \Gamma_G}{\Gamma_2 + \Gamma_G}\right)^2 \qquad [26]$$

where Γ is the diameter of spherical molecule model. The equation is in relatively good agreement with observation. The interpretation of the equation indicates that not only the relative mass difference between isotopic water molecules but also the displacement of the center of gravity of the water molecules on isotopic substitution plays an important role in determining the kinetic fractionation effect.

This kinetic effect, however, appears to be a unique characteristic of evaporation. No kinetic effect associated with condensation processes has been reported (16). Experiments show that deuterium content is much less sensitive to kinetic effect than the ^{18}O component since αD deviates no more than 15% from equilibrium value, whereas $\alpha^{18}O$ deviates 200% (16).

6. Natural Isotopic Variation in Meteoric Water

The study of natural isotope abundances in meteoric water is very important because meteoric water is involved in almost all biochemical reactions occurring on land. The isotopic composition of substances formed in such reactions is influenced by the isotopic composition of the source water. The isotopic composition of meteoric water at a given site depends not only on temperature but also on the thermodynamic conditions prevailing during evaporation and condensation and on the initial isotopic composition of the source water.

Condensation is the most important process that determines the isotopic composition of precipitation. The first small amount of water condensed from the vapor in equilibrium with the source water will have essentially the same isotopic composition as the source water from which the vapor was derived. By further condensation the vapor becomes progressively more depleted in heavy components and so does the newly formed condensate from the vapor. Dansgaard (16) has examined various condensation processes and the corresponding isotopic fractionation. If condensation occurs in a closed two-phase system with equilibrium between the liquid and vapor phases at any stage, the isotopic composition of liquid and vapor can be expressed as

$$\delta_v^* = \frac{1}{\alpha_o \alpha} \cdot \frac{1}{\varepsilon F_v + 1} - 1 \qquad [27]$$

$$\delta_c^* = \frac{1}{\alpha_o} \quad \frac{1}{\varepsilon F_v + 1} - 1 \qquad [28]$$

where $\varepsilon = \frac{1}{\alpha} - 1$, $\alpha_o = \alpha$ at the beginning of the processes, and F_v = the remaining fraction of the vapor phase (15). In the case of isothermal condensation,

$$\lim_{F_v \to 0} \delta_c^* = \frac{1}{\alpha_o} - 1 \qquad [29]$$

$$\lim_{F_v \to 0} \delta_v^* = \frac{1}{\alpha_o^2} - 1 \ . \qquad [30]$$

α values of condensate and vapor formed in isothermal processes are shown as two practically straight lines in Fig. 2. If the condensation is caused by cooling, α will increase as the process proceeds. The isotopic compositions for such cases are shown by the dashed curves in Fig. 2.

Rayleigh condensation is a slow process with immediate removal of the condensate from the vapor after formation. Under Rayleigh conditions, condensates and vapor will change as

$$\delta_c = \frac{\alpha}{\alpha_o} \ F_v^{\alpha_m - 1} \qquad [31]$$

$$\delta_v = \frac{1}{\alpha_o} \ F_v^{\alpha_m - 1} - 1 \qquad [32]$$

respectively, where α, α_o and α_m are fractionation factors at momentary temperature t, initial temperature t_o and average temperature $(t+t_o)/2$, respectively. These are shown as δ_c and δ_v in Fig. 2. In contrast to condensation under closed two-phase system conditions, fractionation

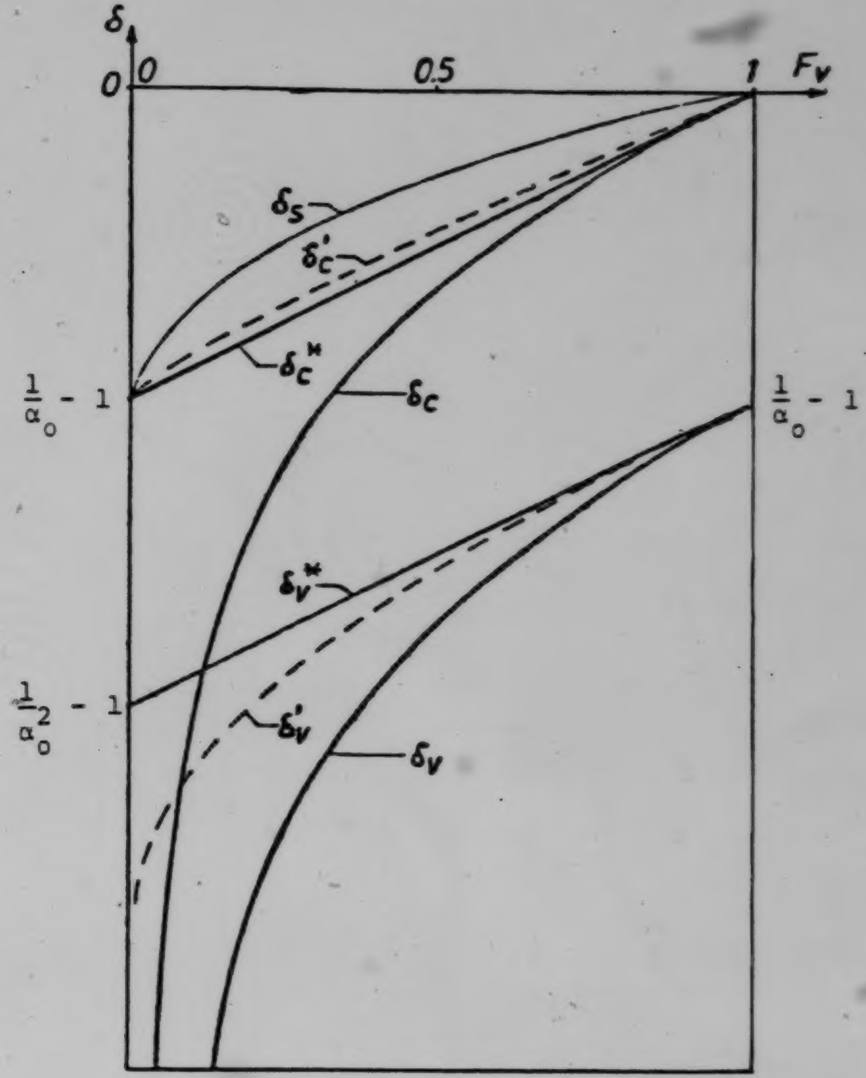


Figure 2. Isotopic fractionation of the remaining vapour and of the condensate as a function of the remaining fraction, F_v, of vapour (16).

$\delta_v^*(\delta_c^*)$ = equilibrium between the total liquid and vapour phases during isothermal condensation

$\delta_v'(\delta_c')$ = same as above during condensation by cooling

$\delta_s(\delta_v)$ = sublimation by cooling. δs is the mean composition of the solid phase

$\delta_v(\delta_c)$ = sublimation or condensation by cooling under Rayleigh conditions. δc is the composition of newly formed condensate.

under Rayleigh conditions increases drastically as condensation proceeds. In nature, however, exchange will more or less smooth out the phenomenon and the actual isotopic compositions of water will follow curves in between the two extreme δ'_c and δ_c curves. In fact, isotopic vapor pressure data collected by Craig et al (14) show that

$$\delta D/\delta\, ^{18}O = 8 \text{ at } -10°C$$

$$\delta D/\delta\, ^{18}O = 5 \text{ at } 100°C$$

corresponding to Rayleigh processes at liquid-vapor equilibrium. Craig (12) has obtained essentially the same relationships between δD and $\delta^{18}O$ with water samples from various localities.

Evaporation at the site of precipitation is also important in determining the final composition of precipitation. During their fall from cloud to ground the rain drops are subjected to evaporation and exchange with the environmental vapor. The evaporation is relatively high in a dry region and probably proceeds under non-equilibrium conditions (21). When the humidity is high exchange between the environmental vapor and the newly formed condensate will possibly be the dominating factor (42).

A number of researchers have studied the fractionation occurring during evaporation and condensation of water vapor as it is carried in an air mass.

1. Epstein et al (27) found that oceans, being large

reservoirs of water, have a fairly uniform isotopic composition disregarding those parts which are directly mixed with fresh water.

2. The deuterium and oxygen 18 concentrations of meteoric water usually vary in parallel (40,27,12,16). In general, deuterium and oxygen 18 variations in water from various rivers, lakes, rain and snow follow a relationship

$$\delta D = 8\delta^{18}O + 10 \qquad \text{(Craig, 12)} \qquad [33]$$

except for water from closed basins in which evaporation is the dominant factor governing the isotopic relationship.

3. The heavy isotope content in precipitation decreases as the condensation temperature drops. This is related to several factors such as latitude and altitude (16).

4. There is also a seasonal effect on the isotopic composition of rain water; isotopic composition is lighter in winter precipitation than in summer precipitation because of cooler condensation temperatures in winter (54).

7. Isotope Thermometry

The isotopic composition of molecules formed in natural processes depends not only upon the temperature of the system but also on the overall isotopic composition of fluids and other solid phases in equilibrium. When two solid phases formed in equilibrium are considered the isotopic fractionation between the two will be determined only by temperature and it will be independent of the presence or quantity of any other substances. This suggests that for a pair of co-existing molecules, if the relationship between the fractionation factor and the temperature is known (either by experiment or theoretical calculation), the measured fractionation between the pair will yield the temperature of last equilibrium. This is the basic principle behind the so-called "thermodynamic thermometry" of stable isotopes (65). Most of the geothermometers dealing with the formation temperature of minerals belong in this category. For example, Bottinga et al (7) found the fractionation factors between quartz and magnetite in the temperature range 600°C to 850°C to be related by

$$\delta^{18}O_Q - \delta^{18}O_M = 5.57 \times 10^6 T^{-2} \qquad [34]$$

where Q = quartz, M = magnetite and T = absolute temperature.

In practice, instead of measuring absolute isotope abundance ratios, it is customary to measure the isotope ratio relative to a standard and to quote the result as a δ value defined by

$$\delta = \left(\frac{R_x}{R_s} - 1 \right) \times 1000 \qquad [35]$$

where R_x = isotope ratio of the unknown sample and R_s =

isotope ratio of the standard sample. The most commonly used standard is SMOW (STandard Mean Ocean Water) whose $\delta^{28}O$ and δD values are set equal to zero. A positive value for δ means the sample is enriched in the heavy isotope relative to sea water and a negative δ value means the sample is depleted in the heavy isotope relative to sea water.

The fractionation factor between two substances A and B can be expressed in terms of δ values:

$$\alpha_{AB} = \frac{R_A}{R_B} = \left(\frac{\delta_A}{1000} + 1\right) \Big/ \left(\frac{\delta_B}{1000} + 1\right).$$

Taking logarithms on both sides,

$$\ln\alpha_{AB} = \text{In}\left(\frac{\delta_A}{1000} + 1\right) - \ln\left(\frac{\delta_B}{1000} + 1\right).$$

If δ_A and $\delta_B \ll 1000$,

$$1000 \ln\alpha_{AB} \simeq \delta_A - \delta_B. \qquad [36]$$

Since most biological reactions occur at near normal environmental temperatures, the fractionation factors involved in such processes are expected to be an inverse function of the absolute temperature:

$$1000 \ln\alpha_{AB} \simeq \delta_A - \delta_B \simeq \frac{a}{T} + b \qquad [37]$$

where a and b are constants. In addition, the total variation of temperature in such reactions is very small (a few degrees). Thus the equation can be simplified to

$$1000 \ln\alpha_{AB} = \delta_A - \delta_B = -CT + b \qquad [38]$$

where C is a positive constant. Thus $\ln\alpha$ turns out to be a negative linear function of temperature. The equation indicates that by measuring the difference in isotopic composition of the two solid phases formed in equilibrium one can deduce the temperature of the equilibrium.

Frequently, it is difficult to find two co-existing solid phases in equilibrium which show a significant temperature-dependent fractionation between them. For example, equilibrium assemblages secreted by organisms, such as calcite, silica, and phosphorite show distinct temperature dependencies of oxygen-isotope fractionation between water and the minerals (13,61,70). Unfortunately, their temperature coefficients are practically identical resulting in no oxygen isotopic fractionation between the co-existing minerals. In such cases, absolute temperature information cannot be obtained and a variety of environmental factors controlling the isotopic composition of the individual substances within the system must be taken into consideration to obtain temperature information. This is the so-called "environmental thermometry", introduced by Lerman (65). Most of the isotope thermometers obtained from biological systems belong in this category.

To determine past climate it is necessary to find natural systems which have recorded the isotope variations in sequence. Such systems must satisfy a number of criteria (48).

1. The isotope variations found within the system must be determined solely by climatic factors or in such a way that non-climatic influences can be filtered out.
2. The isotope record must be permanent. Thus it must be proven that no further fractionation processes such as re-equilibrium or exchange have occurred after the strata have been deposited.
3. The record preserved by the strata should be reasonably complete and continuous.
4. The strata must be datable so that a time scale can be attached to the record.
5. The time resolution of the stratified system must be compatible with the climate information required.

Natural systems which, to a greater or lesser extent, satisfy the above criteria include: deep ocean and lake sediments, polar ice sheets, speleothem deposits, peat deposits, annual coral rings and annual growth rings of trees.

8. Tree Rings

Living organisms consist mainly of the elements oxygen, carbon and hydrogen which are incorporated into their tissues from the environment by metabolic processes.

In consequence, the abundance of stable isotopes of these elements in plant and animal tissues depends both on the isotopic composition of the environment and on the isotope fractionation occurring during photosynthesis and other metabolic processes. The use of biological systems as climate indicators presently presents complex isotopic-geochemical problems. However, high rates of deposition and good time resolution suggest great potential for these methods. Furthermore, biological systems present intriguing possibilities in that they contain three potential isotope indicators which, in any given system, may be independent of one another. These are the isotope ratios $^{18}O/^{16}O$, D/H and $^{13}C/^{12}C$, all of which are measurable in a single sample. Thus multiple over-determination of temperature using three isotope indicators may provide an excellent means of cross-checking the result with some level of confidence. It is also possible that non-temperature effects will be demonstrated by the use of multiple thermometers.

One type of sample which appears to be suitable as a recorder of past climate and environmental conditions is matter of vegetable origin, e.g. tree rings, peat, humus, etc. Other samples of organic origin which might also preserve isotope records are those of animals e.g. teeth, antler, bone, skin, eggshell, etc. Animals reflect basically the isotopic compositions of vegetables on which they feed (18,36).

The systems studied to date are tree rings and to a lesser extent peat deposits. Tree rings have great potential as climate indicators for a number of reasons:

1. Annual growth rings of trees provide a time resolution of at least one year.
2. Absolute dating by means of dendrochronology is accurate to one year.
3. A long continuous time period can be investigated. The bristlecone pine chronology from the White Mountains in California, for example, has a length of 7000 years (33).
4. Tree samples are widely distributed throughout almost all continental area.

8.1 D/H Ratios in Tree Rings

The use of the isotopic composition of organically bound hydrogen as a paleothermometer has been recently suggested by Schiegl (90,91) and Libby (66,67). Schiegl et al (92) measured D/H ratios of both marine and land plants and found systematic variations of deuterium content in plants. For example, the hydrogen of both marine and land plants is significantly depleted in deuterium compared to the water in which the plants grew. They also found a systematic relationship between the natural deuterium content of organically bound hydrogen and that of precipitation. Since the deuterium content of precipitation depends mainly on the climate, particularly on mean annual temperature (16), Schiegl (92) concluded that plant material should carry information on past climate. In an attempt to check the conclusion, Schiegl (90) measured the deuterium content of peat from The Netherlands and found a relatively good relationship with mean July temperatures. The method was subsequently applied to growth rings of picea from southern Germany (see Fig. 3). Schiegl (91) found that deuterium variations in tree rings of picea are essentially a function of annual air temperature. Short isotopic variations are partly influenced by changes of relative humidity. On a long term basis, however, it is the mean annual temperature variations which are responsible for deuterium variations.

The isotope thermometer introduced by Schiegl is an example of an environmental thermometer which cannot provide absolute temperatures but yields empirical temperatures which may have been altered by various environmental factors, such as δD values of water, and relative humidity. Nevertheless, this was the first isotope study that examined the possible use of stable isotope data stored in tree rings as a paleothermometer.

Libby (66), on the other hand, utilized a thermodynamic approach. Libby calculated temperature coefficients of several isotope thermometers in bio-organic material using statistical mechanics. The major assumptions made in this calculation are as follows:

1. Cellulose is formed in equilibrium with water.

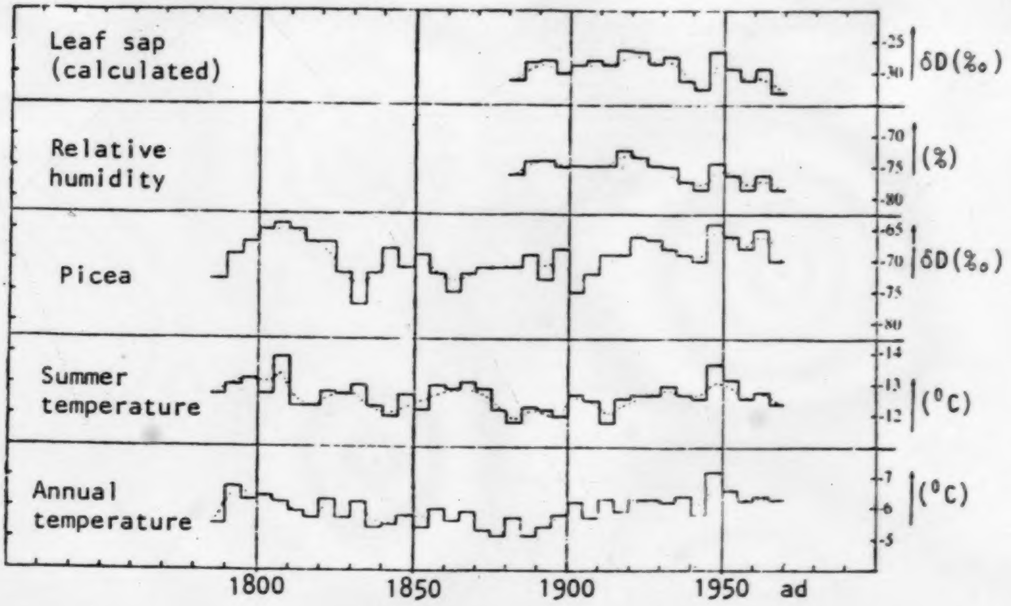


Figure 3. Comparison between summer temperature (May-September), annual mean temperature, deuterium content of Picea, relative humidity (May-September) and the calculated deuterium content of the leaf sap all in the 5-year means. Dashed lines indicate the trends in the histograms (91).

2. Only the internal vibrational frequencies and the bending frequencies associated with rotational energies are included in the calculation of partition functions.
3. The internal vibrational frequencies used were assumed to exist in cellulose molecules. The actual presence of such frequencies and any other possible frequencies were not verified experimentally.

The fractionation factors and their temperature coefficients thus calculated are shown in Table 3.

Table 3. Fractionation factors computed for isotopes of hydrogen and oxygen in cellulose (66)

Fractionation α	Temperature °K		Temperature coefficient °/oo/°C
	273	298	
$\alpha^{18}O_2$-cellulose	1.1624	1.1863	0.96
$\alpha^{18}O_{CO_2}$-cellulose	1.1200	1.1484	1.14
$\alpha^{18}O_{H_2O}$-cellulose	1.1725	1.1956	0.92
αD_{H_2O}-cellulose(OH)	1.5881	1.5983	0.4
αD_{H_2O}-cellulose(CH)	1.8095	1.8606	2.0

For example, the temperature coefficient of the fractionation factor between water and carbon-bound hydrogen in cellulose was found to be 2°/oo/°C. Libby has compared this value with the measured temperature coefficient of 3°/₀₀/°C obtained by Schiegl (85) for a peat deposit in The Netherlands. However, this comparison is not valid because the value 2°/₀₀/°C represents the temperature coefficient of hydrogen isotopic fractionation between water and CH groups in cellulose whereas the value 3°/₀₀/°C is the temperature coefficient of deuterium content in whole wood.

For the purpose of comparison, the properly calculated temperature coefficient of δD values of cellulose would be 1.1°/₀₀/°C provided that the δD of water is constant. This temperature coefficient appears to be too small to be used as a thermometer because the average precision of δD measurement is about ±2°/₀₀. Furthermore, the magnitude of the variation in annual mean air temperature is generally small. For example, the total variation in mean annual temperature in southern Germany for the period Schiegl examined is less than 2°C. The corresponding δD variation would then be 2.2 °/₀₀ which is within the random experimental error. Therefore, the small temperature coefficient calculated by Libby may mean that cellulose-water in tree rings cannot be used as a thermodynamic thermometer. However, D/H ratios preserved in tree rings may provide an environmental thermometer. It is apparent that more work should be done to examine this temperature-dependent fractionation of hydrogen isotopes in tree rings.

To compensate for the limitation of tree rings as a thermodynamic thermometer, Libby (66) has suggested the use of multiple thermometry in tree rings. In photosynthetic production of bio-organic materials, all three elements C, H and O may yield independent thermometers. In contrast to the relationship between D and ^{18}O in meteoric water, the three elements are not functionally related in photosynthesis. The multiple overdetermination of temperature using a set of thermometers may be useful for cross checking the isotope result. In particular, it may be possible to detect some effects that are not caused by temperature and to filter out non-climatic effect.

Libby et al (67) measured the isotope ratios of C, H and O in tree ring sequence of a German Oak and correlated them with the existing weather records from England, Basel and Geneva to evaluate the empirical temperature coefficients. The temperature coefficients are listed in Table 4.

The isotope data along with winter temperatures recorded in England are plotted in Fig. 4. Unfortunately, it is impossible to compare these results with others because the isotope ratios were measured relative to an internal working standard instead of an internationally accepted standard. The magnitude of the temperature coefficients obtained is large. This was attributed to the fact that the temperature coefficient is a combination of equilibrium fractionation during photosynthesis, and both equilibrium and non-equilibrium fractionation during precipitation. Epstein et al (30), however, demonstrated that the combined effect of thermodynamic and environmental temperatures could not account for such large temperature coefficient.

Table 4. Temperature coefficients computed from measured isotope ratios and weather records (67)

Isotope ratio	Temperature coefficient	No. of Samples
$^{18}O/^{16}O$	$(5.29 \pm 0.68) T_{England}$ °/₀₀/°C	68
	$(2.91 \pm 0.41) T_{Basel}$ °/₀₀/°C	56
	$(2.86 \pm 0.52) T_{Geneva}$ °/₀₀/°C	56
D/H	$(89.5 \pm 16.0) T_{England}$ °/₀₀/°C	68
	$(67.4 \pm 6.4) T_{Basel}$ °/₀₀/°C	56
	$(71.4 \pm 8.2) T_{Geneva}$ °/₀₀/°C	56

Nevertheless, subsequent work done by Libby et al (69) demonstrated qualitative correlation between air temperature and stable isotope ratios in tree rings. Their subjective choice of temperature data for correlation purposes was again criticized by Epstein (30).

The isotope results of Schiegl and Libby were obtained using whole plant material and suffered from several complications. Plants are extremely complex chemical systems. Wood for instance consists of approximately 40-50% cellulose and 20% hemicellulose, the rest being mainly lignin (30%)

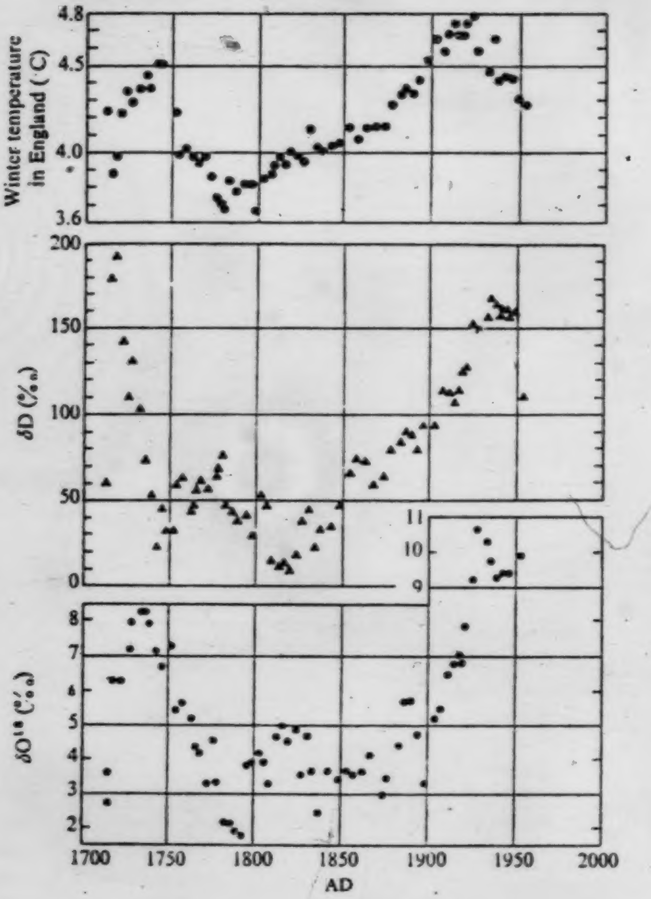


Figure 4. Deuterium and oxygen ratios in German Oak compared with English winter temperature (30-year running averages) (67).

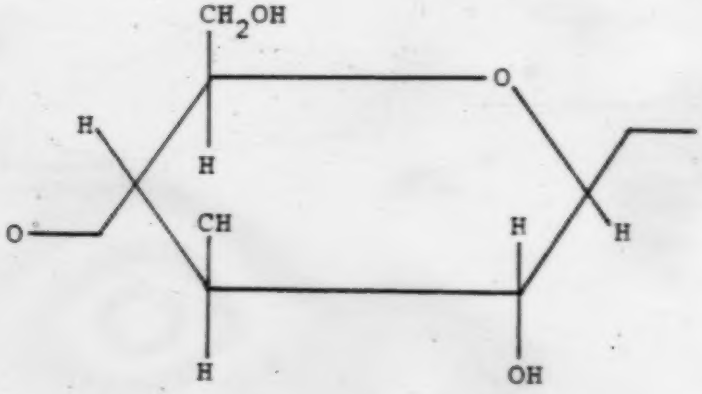
with small amounts of other material (see Table 5).

Table 5. Average % chemical composition of softwoods and hardwoods (95)

Component	Softwoods	Hardwoods
Cellulose	42	45
Hemicellulose	27	30
Lignin	28	20
Extractives	3	5

Isotopic differences between chemical substances in plants may affect the D/H ratio determined for the total system. Smith and Epstein (93) found that the lipid fraction of plant material is approximately 100% depleted in deuterium with respect to the plant total hydrogen. The lignin fraction of whole wood is some 10°/₀₀ lighter in ^{18}O than the cellulose fraction (51). Furthermore the percentage composition of chemical substances is not climate dependent (51). To resolve the problem associated with chemical heterogeneity of plants, Epstein et al (31) and Wilson et al (107) used a single substance, namely cellulose, instead of whole wood.

Cellulose is the only substance in plants whose chemical structure is relatively well defined. The definition of cellulose has been a point of discussion among workers in the field. There is a general agreement that cellulose is composed predominantly of β-linked D-glucopyranose units (53). Thus, extraction of cellulose in the pure form from woody tissue is physically impossible. The term cellulose in this study, therefore, is referred to as α-cellulose which is alkali-resistant cellulose retaining 0.5-1.5% of non-glucan carbohydrates. The molecular structure of cellulose can be represented by



In a cellulose molecule, the hydrogen atoms fall into 2 groups. One is carbon-bound hydrogen (CH) and the other is oxygen-bound hydrogen (OH) comprising 70% and 30% respectively. The bond strength of a CH bond is greater than that of an OH bond. Thus their hydrogen isotopic compositions may reflect two different fractionations. An additional complication is the problem of isotopic exchange of hydrogen. Carbon-bound hydrogen in cellulose is non-exchangeable under normal conditions whereas the hydroxyl hydrogens exchange readily (43,72). In addition, fine structures of cellulose also influence the hydrogen isotopic composition of cellulose. Cellulose consists of highly ordered crystalline as well as disordered amorphous regions (59). With different celluloses, the relative amount of material in the crystalline and in the amorphous regions, as well as the degree of order within the crystalline region, are not the same. Infrared spectral analyses by Mann et al (72) show that about 25% of the hydroxyl groups in cellulose are in the crystalline region and that about 30% of this hydroxyl hydrogen together with all the hydroxyl hydrogen in amorphous regions exchange with water. In other words, 25% of the total plant hydrogen in cellulose comprising 22% from amorphous OH group and 3% from crystalline OH group exchanges with the hydrogen in water.

To resolve the problem of exchangeable hydrogen in cellulose, Wilson et al (10) equilibrated the hydroxyl hydrogen with water of known isotopic composition. Using this technique, they analyzed D/H ratios in the cellulose from a Monterey Pine (Pinus Radiata) growing in Hamilton, New Zealand (see Fig. 5). They found that tree rings formed in summer were more depleted in deuterium than winter rings. Since the isotopic composition of the cellulose varies in the opposite direction to the isotopic composition of precipitation, they concluded that the D/H ratio of cellulose in the wood of Monterey Pine changes with temperature due to the temperature effects on biochemical reactions leading to cellulose. Taking into account the isotopic variations of the atmospheric precipitation, they obtained a temperature coefficient -5°/₀₀ per °C.

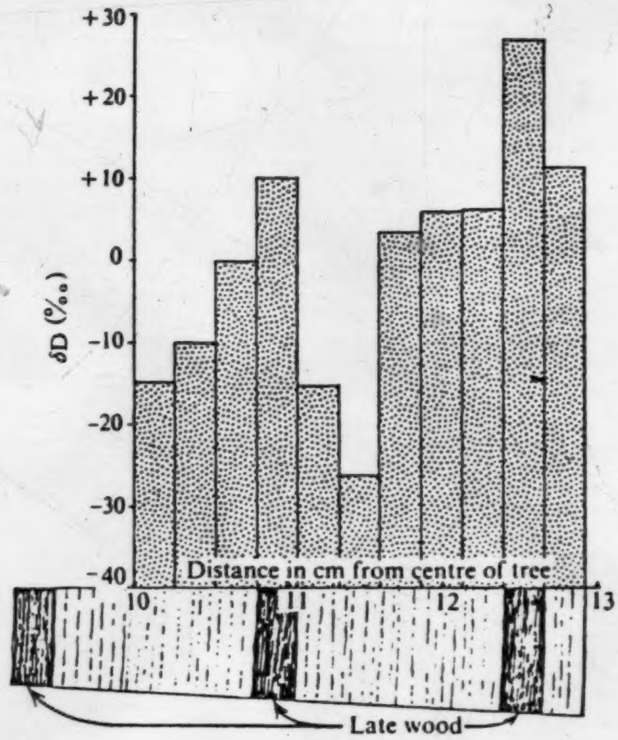


Figure 5. Variation in isotopic ratio of the C-H hydrogens in cellulose across tree rings (107).

This work has been criticized in that the negative temperature coefficient is opposite to that expected from thermodynamic arguments. According to thermodynamics, the isotope fractionation in a chemical reaction normally decreases as the temperature of the system increases. To satisfy the thermodynamic condition, the D/H ratio of cellulose should increase as the temperature of the system increases because the hydrogen isotopic composition of meteoric water is a positive linear function of air temperature. There are several possible explanations for this contradiction. The phenomenon may be explained in terms of a time lag between the actual photosynthesis and cellulose deposition. A conifer manufactures photosynthates at all times of the year except when climatic conditions are unsuitable. These photosynthates are stored for short or extended periods before they are laid down as wood. Thus the cellulose in late wood of Monterey Pine might have been produced in summer instead of winter and thereby show a high D/H ratio reflecting the D/H ratio of summer precipitation. When this time lag is considered, the system may show a positive temperature coefficient. Another possible explanation was given by one of the authors. Wilson et al (107) assumed that the fractionation between the hydrogen of the water and the OH hydrogen of the cellulose was small. Wilson (106), however, pointed out that the fractionation may be very large, perhaps due to the extensive hydrogen bonding in the cellulose. The fractionation must be of the order of 200°/∘∘ such that the OH hydrogen is enriched in deuterium. The corresponding temperature coefficient is in the order of 10°/∘∘/°C. Thus if the crystalline OH hydrogen atoms have a very large negative temperature coefficient, outweighing the positive temperature coefficient of the CH hydrogen, the net result would be a negative temperature coefficient. However, the temperature coefficient found by Libby for the fractionation between OH hydrogen in cellulose and hydrogen in water had not only a very small magnitude but also opposite sign.

On the other hand, Wilson (106) proposed a possible thermodynamic thermometer in tree rings, which utilizes both CH and OH hydrogen of cellulose. He hypothesizes that the OH hydrogen in the crystalline region of cellulose may provide a sensitive paleothermometer which would be independent of the cell water composition. The isotope ratio of OH hydrogen could be determined by measuring the δD of non-exchangeable CH hydrogen by replacing OH hydrogen with $-NO_3$ groups and non-exchangeable hydrogen (both CH and OH) determined by the equilibration method. Using this technique, Friedman et al (41) calculated δD values of OH hydrogen in cellulose and correlated it with the altitude of growth sites to find the temperature relationship. The non-exchangeable OH hydrogens thus obtained were more enriched in deuterium than sap water by as much as 650°/∘∘ indicating that the fractionation between OH hydrogen in cellulose and hydrogen in water is large. In contrast to Wilson's prediction however, temperature dependence of the fractionation was not detected, which may be in keeping with Libby's prediction. It is of interest to note from the result of Friedman et al (41) that non-exchangeable CH hydrogen in cellulose is more enriched in deuterium than that of stream water by about $30^{\circ}/_{\circ\circ}$. In one case δD values of CH hydrogen were found to be even higher than that of sap water. These are opposite to the results obtained by Epstein et al (31) and White et al (103) in which CH hydrogen, regardless of temperature and relative humidity, is more depleted in deuterium than environmental water and sap water by about $22^{\circ}/_{\circ\circ}$. However, it is apparent that further work with a well defined technique is required to resolve this contradiction.

A second method of dealing with the exchangeable hydrogen in cellulose has been reported by Epstein (31). Plant samples were nitrated and pure cellulose nitrate was extracted by acetone dissolution. Subsequent tests of the nitrated product have demonstrated that the nitration extraction procedure eliminates the OH hydrogen in the cellulose but does not alter the D/H ratio of carbon-bound hydrogen.

Using this technique, they measured the D/H ratio of non-exchangeable hydrogen in cellulose extracted from aquatic and land plants. They found that plant-extracted cellulose nitrate D/H ratios are systematically related to the D/H ratios of the associated environmental water (see Fig. 6). The equation thus obtained is

$$\delta D_{cell} = 0.97\ \delta D_{w} - 22°/_{\circ\circ}$$

where cell and w represent cellulose and environmental water respectively.

From the fact that the overall relationship is linear with slope close to unity, they concluded that D/H ratios of cellulose in tree rings reflect mainly the D/H ratios of environmental water. In support of this, White et al (103) found a similar linear relationship between the D/H ratios of the non-exchangeable hydrogen in tree cellulose and the model-calculated D/H ratio of tree sap for eastern white pines:

$$\delta D_{cell} = \delta D_{sap} - 21 \qquad [39]$$

where sap represents sap water.

The slope and the intercept of this equation are the same as those of the equation obtained by Epstein et al (31) despite the fact that White used sap water whereas Epstein used environmental water. This may indicate that absorption of water by plant roots involves no fractionation. The temperature distribution over much of the earth's surface has been correlated with the pattern of D/H and $^{18}O/^{16}O$ variations in meteoric water (16). Thus natural systems which record the variations with time of

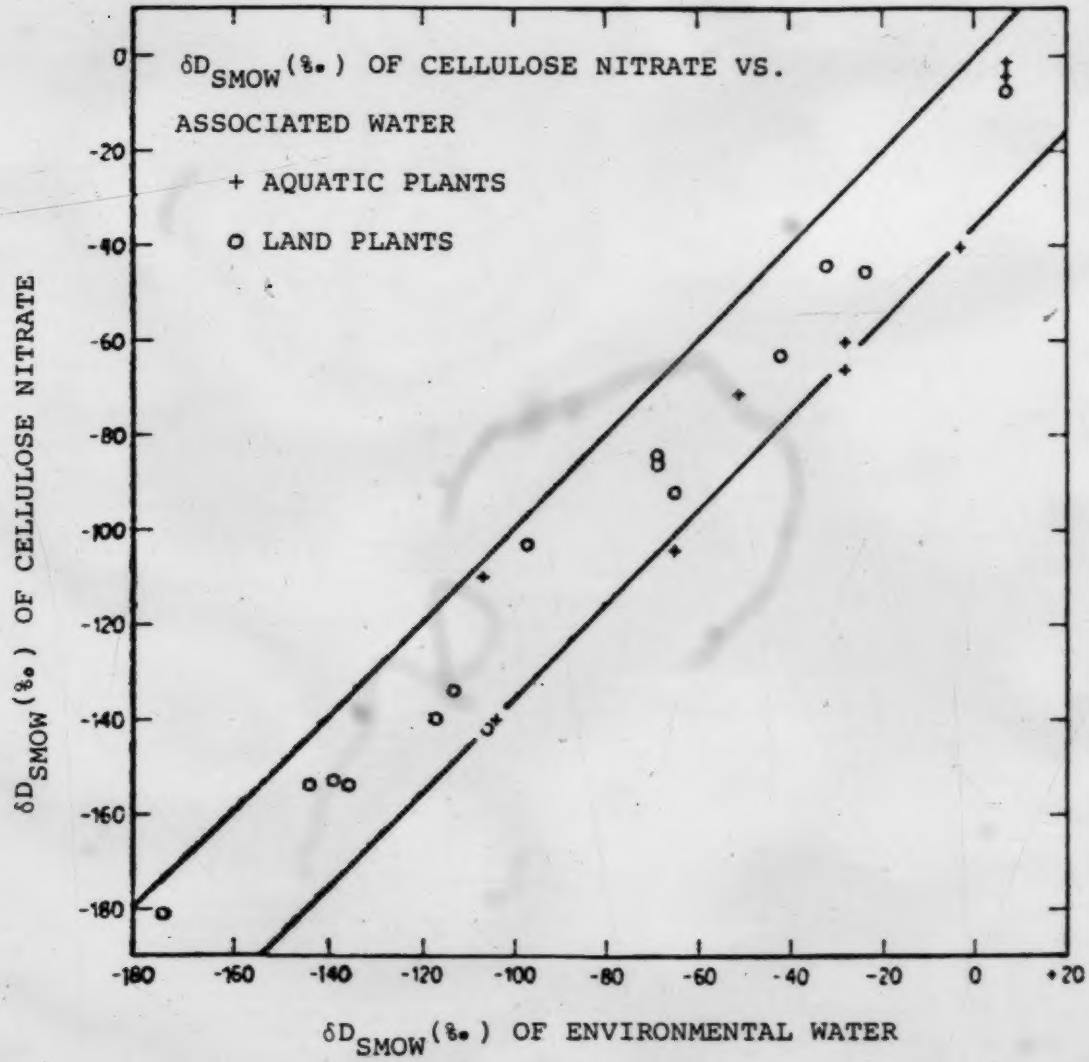


Figure 6. The relationship between the δD of the cellulose nitrate extracted from plant material and the δD values of meteoric waters associated with the plants (31).

the D/H or $^{18}O/^{16}O$ ratios of meteoric waters at a given site should preserve a record of past temperature variations at the site.

Epstein et al (31) also found non-systematic but significant differences between the values of plant total hydrogen and non-exchangeable carbon-bound hydrogen (see Fig. 7). Unlike non-exchangeable carbon-bound hydrogen, plant total hydrogen has a poor functional relationship with environmental water. They attributed this poor correlation to the effect of chemical heterogeneity on δD value of plant material.

Epstein et al (29) subsequently applied this technique to paleoclimate reconstruction. Measuring D/H ratios of non-exchangeable hydrogen in cellulose, they obtained a δD record spanning 1000 years from two bristlecone pines, whose growth periods overlapped by about 100 years, from the White Mountains in California. This record is compared with Lamb's winter temperature curve for central England and ring width variations in a bristlecone pine from the White Mountains in California (see Fig. 8). There is qualitative agreement between records. For example, all four records in Fig. 8 show a cooling period between 1450 and 1800 AD which is often called "The Little Ice Age". Epstein et al (29) also obtained δD records from a Scots pine located near Loch Affric, Scotland (see Fig. 9). The average δD value of the scots pine is about -60°/₀₀ as compared to -95°/₀₀ in the bristlecone pine for the corres-

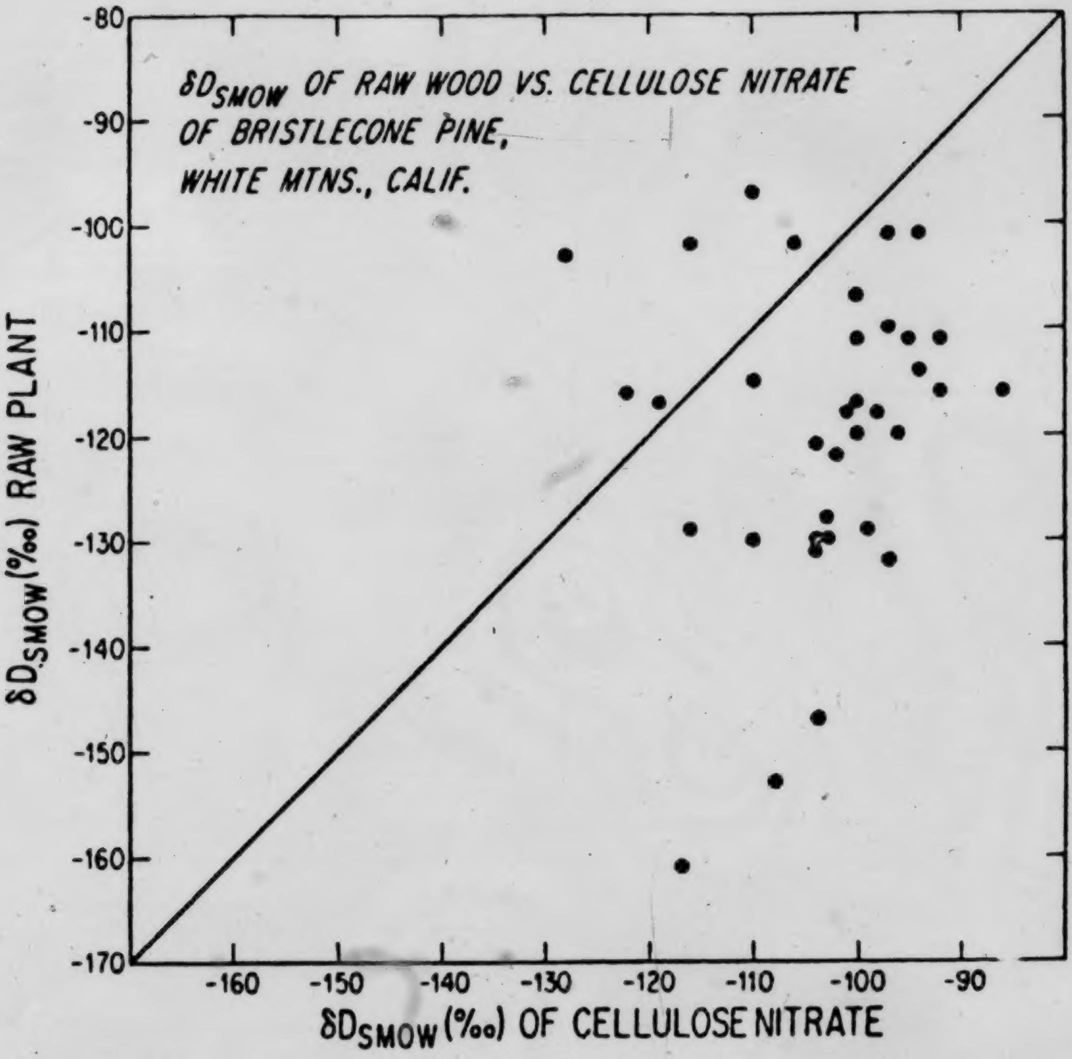


Figure 7. The relationship between the δD values of raw wood from a single bristlecone pine and the corresponding cellulose nitrate extracted from the raw wood. Each point represents a different 10-year interval of growth (31).

ponding time interval. The difference is in keeping with the difference of the δD values of meteoric waters in their respective localities, -42°/₀₀ for Loch Affric and about -100°/₀₀ for the waters in the bristlecone pine area. A comparison of the 40 year running average of the δD values of the two pines produces a relatively good linear relationship which leads Epstein to suggest that long term isotopic climatic trends, as recorded by the two species of pine are similar, while the short term fluctuations are not related. Epstein et al (29) conclude that widely different types of climatic environments produce δD trends which may reflect large scale long-term climatic changes, superposed upon which there may exist short term δD variations which could reflect temperature changes over a restricted local area.

The ultimate purpose of paleoclimate reconstruction is to identify possible periodicities in climate variations and the causes of such variations. Libby et al (68) calculated climate periods from stable isotope ratios of oxygen and hydrogen measured in a Japanese cedar spanning 1800 years. These climate periods were compared with those from 800 year Greenland ice (17) and with periods computed from the tidal stresses of the Sun-Moon-Earth system. According to Libby a good correlation was evident. Also, Fourier analysis, done by Epstein et al (29) on the isotope data of the bristlecone pines from the White Mountains, showed a 22 year periodicity in the power spectrum. They related it to the 22 year drought cycle in the midwestern

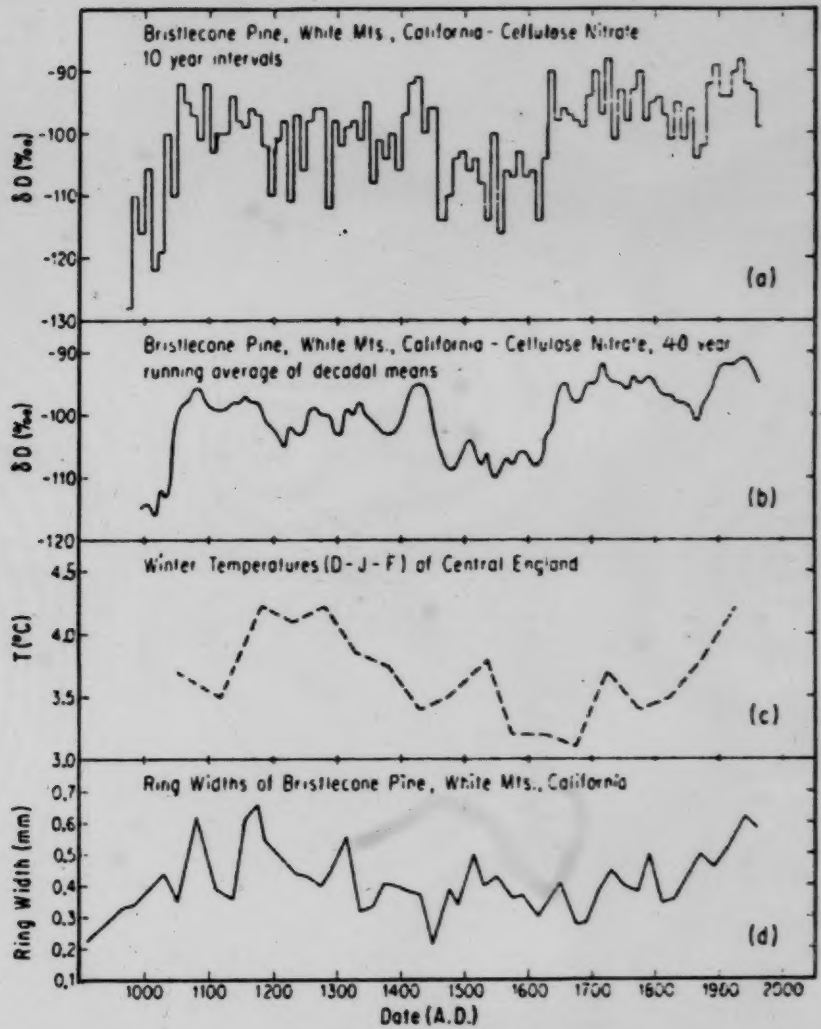


Figure 8.

a) The δD record of isotopically non-exchangeable hydrogen of cellulose (cellulose nitrate) in two bristlecone pines which grew in the White Mountains of California.

b) The 40 year running average of the δD record.

c) Winter temperatures of Central England (Dec-Feb) from a variety of indicators.

d) Absolute ring width variations in a bristlecone pine from White Mountains, California (63,29).

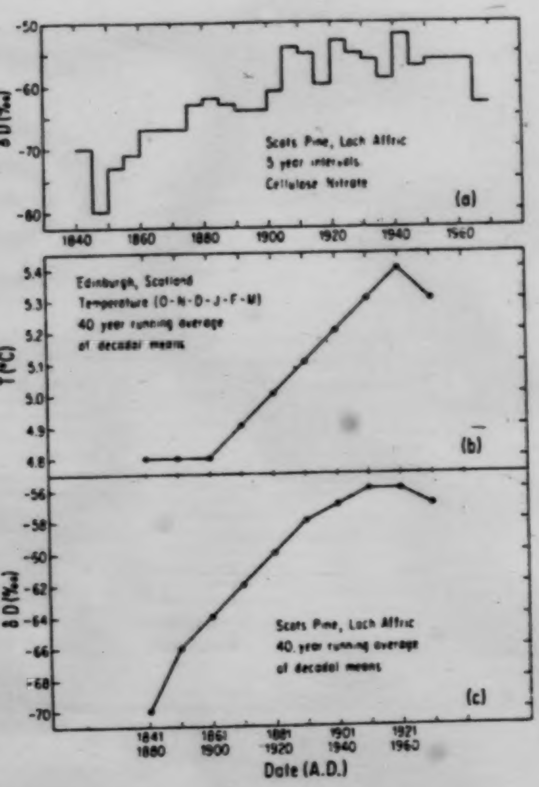


Figure 9.

a) The δD record of cellulose nitrate from 5-year segments of a Scots pine growing in Scotland.

b) The 40-year running average of decadal means for winter temperatures (October through March) of Edinburgh, Scotland.

c) The 40-year running average of decadal means of δD of the Scots pine (29).

U.S. (84). The 22 year cycle of drought in turn has been attributed to the Hale sunspot cycle which seems to occur at about 22 year intervals (76). The validity of the 22 year cycle in the power spectrum of the bristlecone pine isotope data, however, is not firmly established due to possible aliasing problems. In any time series analysis of discrete experimental data, the maximum statistically meaningful frequency is the Nyquist frequency defined by

$$\nu_n = \frac{1}{2\Delta T} \qquad [40]$$

where T is the sampling interval (60). The sampling interval chosen by Epstein et al (29) was 10 years. The corresponding Nyquist frequency becomes 1/20 which is very close to the frequency of the 22 year cycle. Thus, the 22 year cycle may be an artifact of the Fourier analysis of data contaminated with colored noise. Sampling interval of 5 years or less may resolve the presence of the 22 year cycle periodicity if the cycle is indeed real.

8.2 $^{18}O/^{16}O$ Ratios in Tree Rings

Oxygen isotope ratios have been used extensively in paleoclimate studies for a number of reasons. Firstly, there exists a large reservoir of oxygen in the oceans. Secondly, oxygen is very reactive and forms compounds with most other elements and lastly, the natural abundance variations are relatively easy to measure. For example, the natural abundances of the three atmospheric oxygen isotopes, ^{16}O, ^{17}O, and ^{18}O are 99.759%, 0.037%, and 0.2039% respectively (78).

Oxygen isotope ratio measurements on cellulose extracted from trees have been reported by Gray et al (49, 50), and Epstein et al (28). Gray et al (49) have reported ^{18}O values of cellulose from successive 5 year groups of rings in a spruce tree (Piecea glauca) which grew in Edmonton, Alberta (see Fig. 10). A striking linear relationship between $\delta^{18}O$ and mean annual temperature was obtained (see Fig. 11). The temperature coefficient thus obtained is 1.3 0.1°/$_{\circ\circ}$/°C with a correlation coefficient of 0.97. The correlation between $\delta^{18}O$ and mean winter temperature is good whereas it is poor for mean summer temperatures. The best correlation occurs with September to August mean annual temperatures. Since spring and summer are the time most conifers lay down growth rings, the poor correlation between $\delta^{18}O$ and summer temperature requires some explanation. Gray et al (49) proposed two possible explanations for this observation. First, in addition to any temperature effect on the fractionation factor during photosynthesis, there is also variation in the isotopic composition of the source water being used to produce cellulose. They assumed that the cellulose oxygen is derived from CO_2 which undergoes isotopic exchange with H_2O before photosynthesis occurs, and thereby reflects $^{18}O/^{16}O$ ratios of the source water. Thus it is possible that winter precipitation stored as soil water after melting

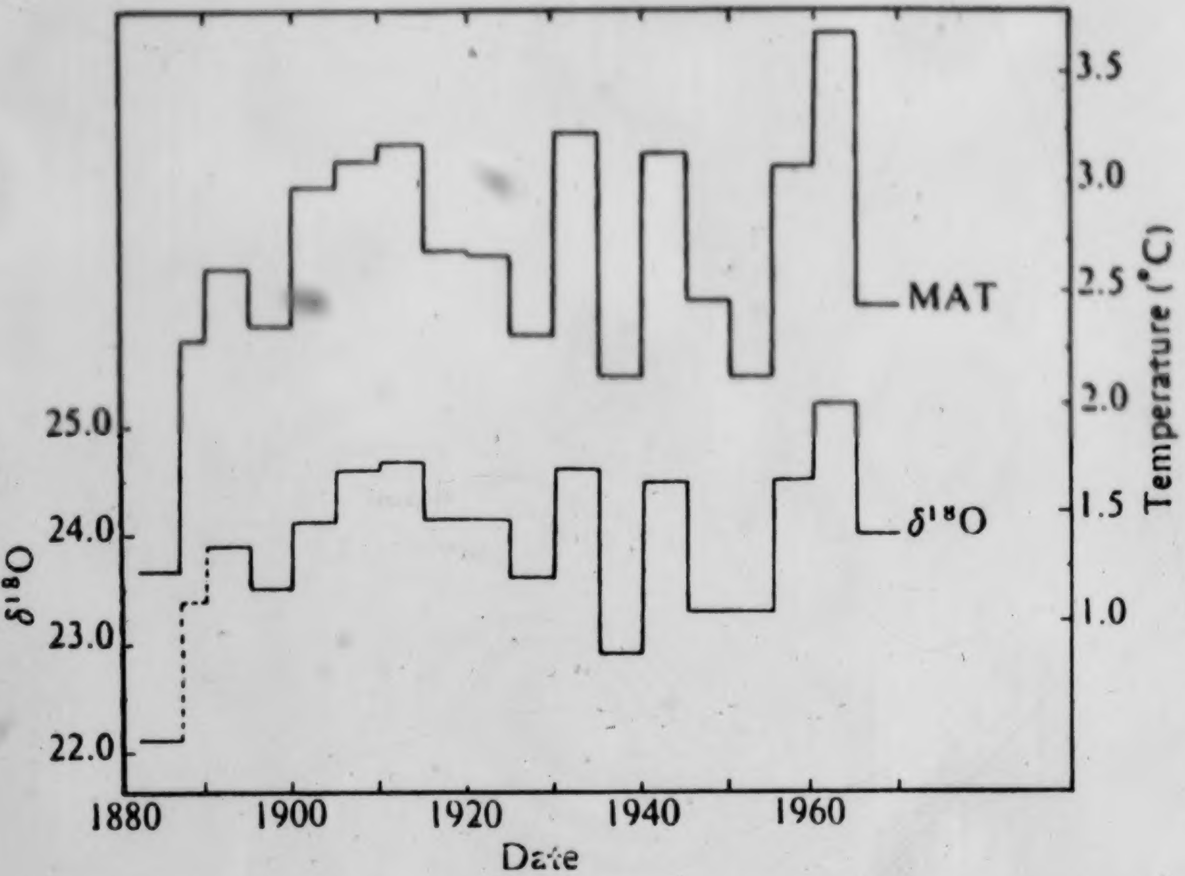


Figure 10. A comparison between the mean annual temperature (MAT) curve obtained from the meteorological data and that obtained from $^{18}O/^{16}O$ ratios of cellulose. (The broken part of the isotope curve is calculated for some out-of-sequence rings that were not analysed (49).

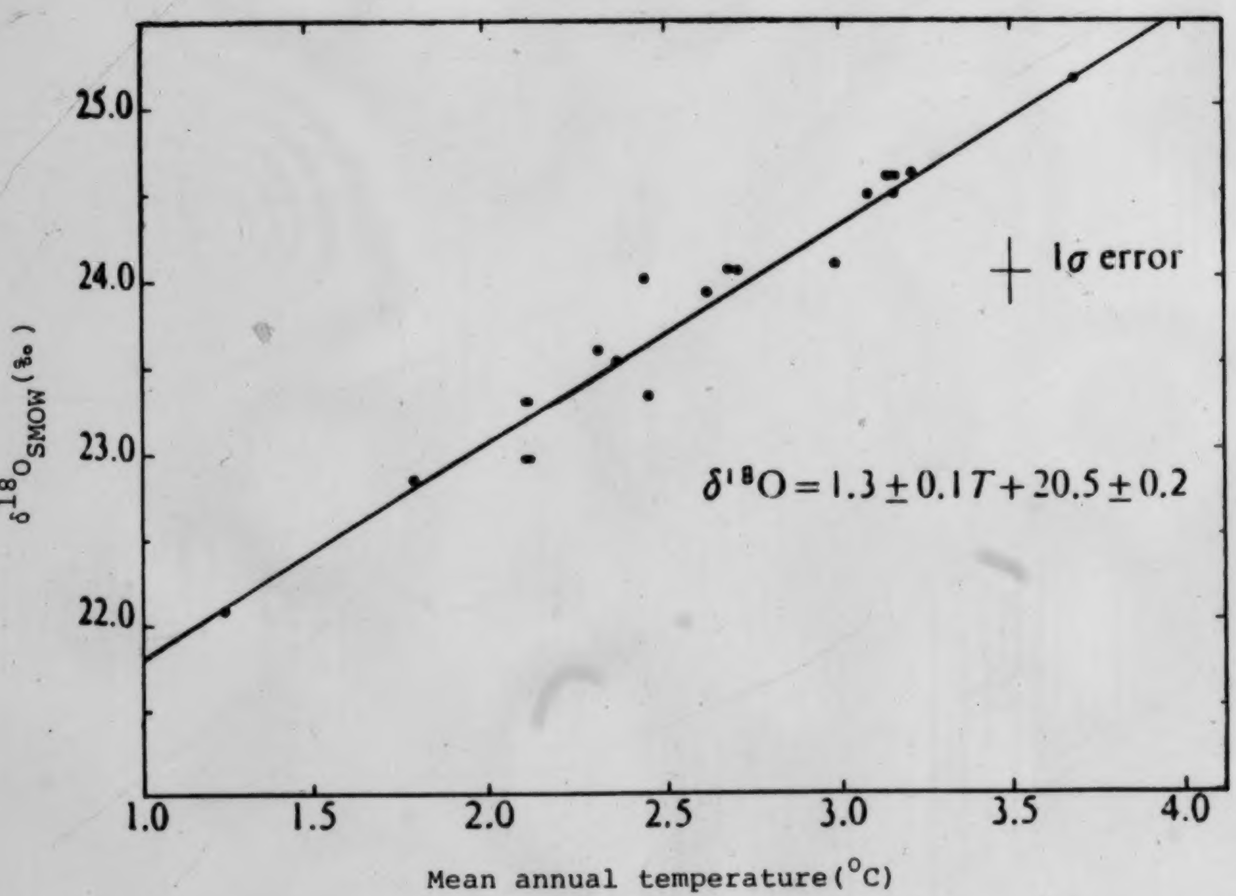


Figure 11. The variation of $\delta^{18}O$ (SMOW) with September to August mean annual temperature for 5-year periods. The equation of the line was obtained by a least-squares fit to the data points (49).

may play a significant role in the production of cellulose during the growing season. Second, it has been shown that net photosynthesis and growth in coniferous trees depend markedly on the production of stored foods. Thus winter temperatures may have a direct bearing on the isotopic composition of the cellulose produced the following year.

This linear relationship between $\delta^{18}O$ of cellulose and mean annual temperature has been criticized by Epstein et al (29) on the basis that $\delta^{18}O$ values of cellulose are affected by evapotranspiration and humidity as well as the $\delta^{18}O$ of the meteoric water. In support of this, Ferhi et al (35), in experiments on bean plants grown under controlled conditions, found that the variation of $\delta^{18}O$ value of cellulose with temperature was very small if not negligible. In a subsequent series of experiments, Ferhi et al (34) concluded that the most important factors in determining $^{18}O/^{16}O$ ratio of plant cellulose were the isotopic composition of water absorbed in the roots and relative humidity. To clear this apparent contradiction, Gray et al (52) measured $\delta^{18}O$ of cellulose for a number of trees from various sites in North America. The overall mean $\delta^{18}O$ and annual temperature data for each site are plotted in Fig. 12. The least squares estimate of the equation defining these data is:

$$\delta^{18}O = 0.54T + 22.9. \qquad [41]$$

These data are compared with $\delta^{18}O$ data obtained from

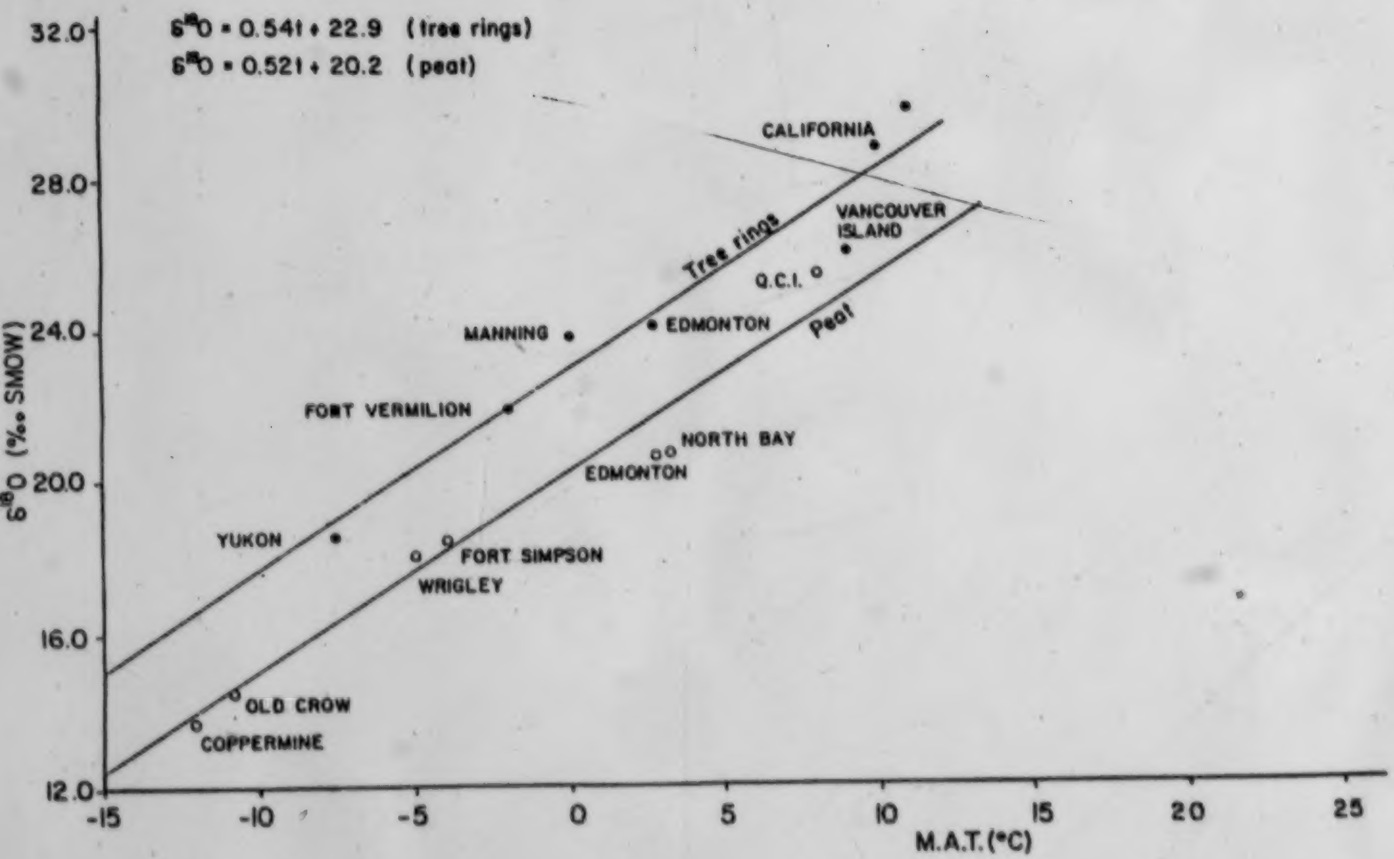


Figure 12. Average isotope value and mean annual temperature (M.A.T.) for each tree and peatbog site (52).

sphagnum moss. The slopes of the two lines are the same within experimental error while the intercepts are different. The fact that $\delta^{18}O$ values in cellulose of tree rings, and sphagnum moss have a linear relationship with air temperature suggests that in many of the regions temperature information is stored in the $\delta^{18}O$ of cellulose. Furthermore, the virtually identical temperature coefficients shown by two different biological indicators suggest that enrichment by evapotranspiration must be relatively constant. Evapotranspiration is known to be controlled mainly by water availability and relative humidity, and is only indirectly related to temperature. The sphagnum moss samples were taken from peat bogs where humidity is generally high and evapotranspiration will be minimized. Thus the displacement of the tree ring line may be explained in terms of enrichment by evapotranspiration. This is possible only if evapotranspiration is assumed to be constant over a wide range of mean annual temperatures. In support of this, a similar functional relationship between mean annual air temperature and $\delta^{18}O$ values of cellulose in trees from various coastal stations in North America has been reported by Burk et al (10). They found a linear relationship between $\delta^{18}O$ of cellulose from tree rings and the latitude of the collection site. When conversion from latitude to mean annual temperature was made, a linear relationship between mean annual temperature and ^{18}O of cellulose was established.

CHAPTER III

EXPERIMENTAL EQUIPMENT AND PROCEDURES

1. Gas Extraction Methods

To determine D/H and $^{18}O/^{16}O$ ratios of organic material, it is necessary to extract the desired elements in a suitable form for mass spectrometric analysis. Oxygen is normally converted to CO_2 while for hydrogen isotopic analysis, hydrogen gas is the most suitable form. The extraction of these gases from organic materials involves various chemical and physical processes which may introduce undesirable secondary fractionation and alter their original isotopic records.

A number of methods for the separate extraction of hydrogen and oxygen from biological material have been described. For the extraction of oxygen, there are a variety of methods (83,96,55). For hydrogen, only one method is generally used. This involves the quantitative conversion of hydrogen in a sample to water followed by reduction of the water over hot uranium metal to hydrogen. These methods are time consuming and often impossible for small samples. In some cases it is desirable to measure both D/H and $^{18}O/^{16}O$ ratios on a given sample. For this reason, an attempt has been made to analyze simultaneously hydrogen and oxygen from a single sample.

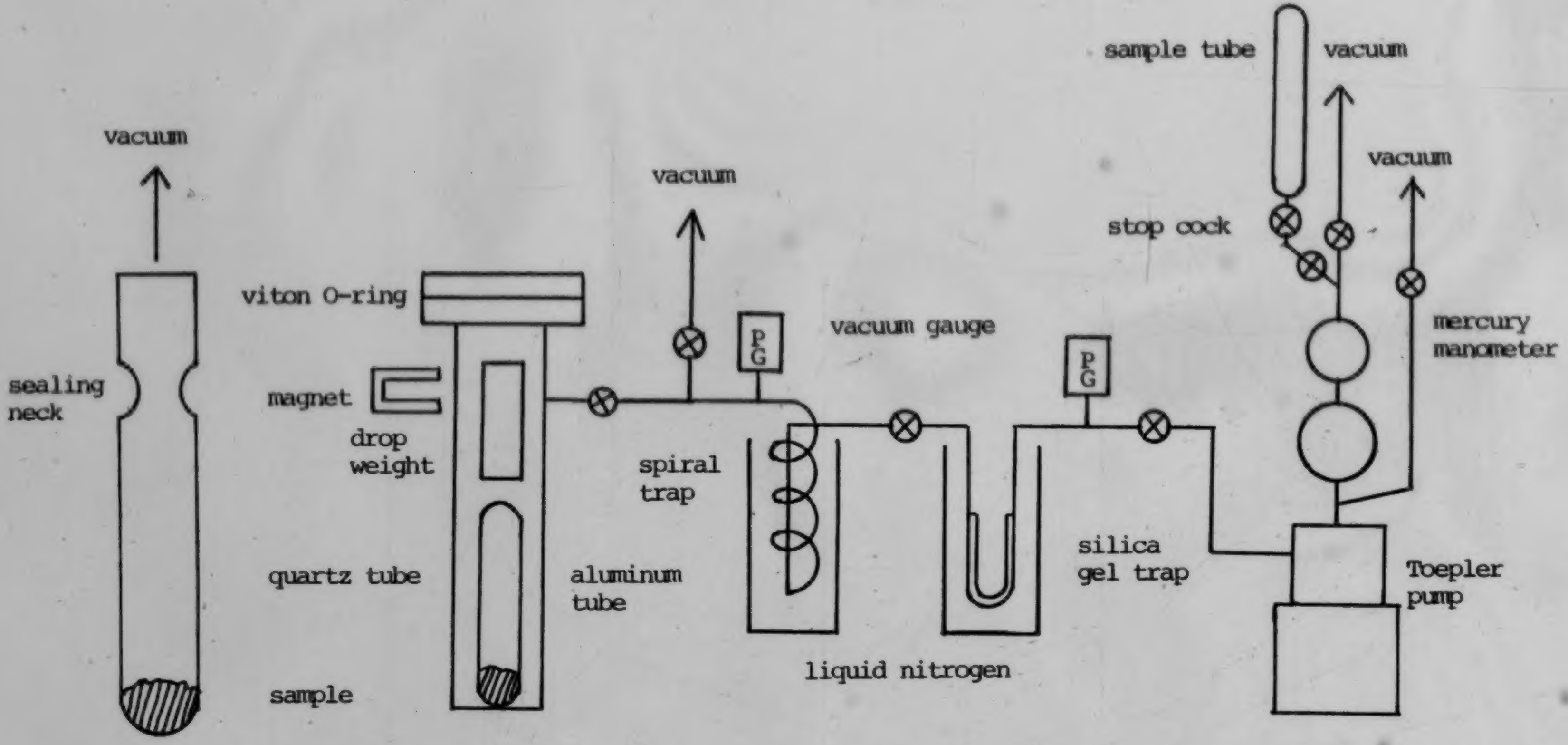
1.1. Pyrolysis of Cellulose in a Quartz Tube

1.1.1. Procedure

The first method used involves the pyrolysis of cellulose in a quartz tube followed by separation of the desired gases from the pyrolysis products. About 30 mg of cellulose is placed in the bottom of a quartz tube 1.1 cm in diameter and 11 cm long. The quartz tube containing the cellulose is then evacuated and sealed with a heating torch. The cellulose in the quartz tube is then pyrolyzed in a furnace at 1200°C for 20 minutes, after which the quartz tube is mounted on the separation line (see Fig. 13). The gases from the pyrolysis are released by breaking the quartz tube mechanically using a drop weight and the individual gases which include H_2, HD, CO_2 and CO are separated by means of chromatographic adsorption.

The gas separation line consists of a spiral trap, a silica gel trap and a toepler pump connected in series. As the gas mixture from the pyrolysis passes the chromatographic columns, CO_2 is held in the spiral trap kept at liquid nitrogen temperature and CO is held in the silica gel trap kept at liquid nitrogen. Hydrogen gas is collected with the toepler pump. The yields of gases thus separated are measured manometrically.

An alternative method for the separation of hydrogen gas from the mixture of gases was used. This


vacuum
sealing neck
viton O-ring
magnet
drop weight
quartz tube
sample
vacuum
P G
vacuum gauge
spiral trap
aluminum tube
liquid nitrogen
P G
silica gel trap
sample tube
vacuum
stop cock
vacuum
mercury manometer
Toepler pump

(A) Quartz Pyrolysis Tube (B) Gas Separation Line

Figure 13. Schematic diagram of gas extraction apparatus. (A) Quartz pyrolysis tube (B) Gas separation line.

involves diffusion of hydrogen through a 75% palladium - 25% silver alloy wall maintained at 500°C. Hydrogen diffuses through the thin wall of the palladium-silver tube and is collected on charcoal at liquid nitrogen temperature. Isotopic ratios of hydrogen and oxygen gases were then determined by mass spectrometry.

1.1.2. Result and Discussion

To determine the optimum experimental conditions for the complete extraction of hydrogen and oxygen from cellulose, samples of Whatman filter paper cellulose were pyrolyzed in quartz tubes under various conditions. The results are shown in Table 6.

The yield of H_2 gas was about 90% of the theoretical value indicating incomplete decomposition of cellulose. To enhance the yield various combinations of reaction time and temperature were employed. At a temperature range of 1280-1320°C, increasing the pyrolysis time produced essentially no increase in the yield of H_2 gas. The variation of temperature in the range of 1280-1320°C appears to have no effect on the yield of hydrogen. A temperature higher than 1300°C is physically difficult to obtain due to fast oxidation of heating elements in the furnace.

Table 6. % Yields of Hydrogen from Pyrolysis of Cellulose

Trial	H_2 Yield %	Temp. °C	Duration minute	D/H ratio °/oo	Cold Trap Temperature °C
19	86	1280	15	-36	-189
20	87	1280	20	-32	-189
21	85	1280	15	-48	-189
22	83	1280	25	-30	-189
23	91	1280	20	-15	-189
26	88	1280	20	21	-128
27	90	1320	20	19	-136
30	94	1320	20	31	- 67
31	96	1320	20	55	- 96
32	100	1320	20	71	- 81
33	102	1320	20	63	- 81
34	102	1320	20	70	- 81
35	94	1320	20	29	-100
36	97	1320	20	116	- 96
37	86	1320	30	4	-189
38	102	1320	35	134	- 81
40	105	1320	20	78	- 81
41	105	1320	60	97	- 81
42	89	1320	35	-15	Pd-Ag
43	90	1320	35	-5	Pd-Ag

The low yield of hydrogen may be due to the production of methane gas during pyrolysis. Mass spectrometric analyses of the pyrolysis products showed the presence of methane including deuterated compounds, such as CH_2D_2, CH_3D, CHD_3 and CD_4. Approximately 10-20% of the total hydrogen in cellulose was in the form of methane at the end of pyrolysis. A similar result has been reported by Holliday et al (58). For thermal decomposition of methane at temperatures up to 1300°C, they found that about 90% of the methane converted to H_2 and the rest remained unaltered. Thermal decomposition of methane at this temperature is believed to take place mainly on surfaces. Thus the reaction is rapid initially and the reaction is virtually complete after 10 minutes at which time the reaction is strongly retarded in the presence of hydrogen gas. The retardation is due to the preferential absorption of hydrogen which protects the surface so efficiently that eventually further decomposition of methane is prevented.

Incomplete conversion of cellulose hydrogen to free hydrogen gas inevitably leads to isotopic fractionation between the hydrogen gas molecules and the compounds containing hydrogen. For example, methane exchanges hydrogen isotopes with hydrogen gas molecules at virtually all temperatures. Therefore the D/H ratio of the hydrogen from the pyrolysis is different from that of the original hydrogen in the cellulose.

The apparently high yields of hydrogen for trial 23-

41 in Table 6 are likely caused by the presence of impurities, mainly methane gas, in the hydrogen gas phase. Depending on the temperature of the silica gel trap, some methane gas may escape the cold trap and mix with hydrogen gas increasing the apparent hydrogen yield. The methane gas, when ionized inside the mass spectrometer loses part of hydrogen gas to form radicals such as CH_3, CH_2, etc. The isotopic composition of the hydrogen thus released from methane is different from that of hydrogen gas. This is illustrated in Table 7.

Table 7. The effect of methane content in hydrogen phase on the overall isotopic composition of hydrogen

Trial	H_2 Yield %	CH_3/H_2 %	D/H Ratio °/₀₀
37	86	0.02	4
35	94	0.25	29
33	102	2	63
34	102	2.2	70
36	97	2.5	116
38	102	3.1	134

The apparent D/H ratios of cellulose hydrogen increase with higher methane content. This indicates that methane is more enriched in deuterium than hydrogen. Unfortunately, the relative amount of methane in the final products of cellulose pyrolysis could not be controlled.

The hydrogen gas from trials 42 and 43 were collected by diffusion through a Pd-Ag tube which results in pure hydrogen. However, they show a rather significant difference (10 °/₀₀) in δD values between the two whereas the difference in hydrogen yield is only 1%. The relative amount of methane in the final products of pyrolysis and the isotopic fractionation associated with it appears to be sensitive not only to the average temperature of the pyrolysis but also to the cooling rate of quartz after the pyrolysis which is almost impossible to control. This may partly account for the poor reproducibility of δD measurement of cellulose. It was also found that oxygen appears to exchange isotopically with quartz (SiO_2). Therefore, the method of pyrolysis of cellulose in quartz tubes cannot be used either for oxygen or hydrogen isotope analyses.

1.2. Pyrolysis of Cellulose in a Nickel Reaction Vessel

The second method of obtaining hydrogen and oxygen for isotopic analysis from organic matter made use of a nickel reaction vessel. The principle of the extraction method is similar to that previously described by Thompson and Gray (91).

1.2.1. The Reaction Vessel

The reaction vessel in Fig. 14 is designed to separate hydrogen gas from carbon dioxide and carbon monoxide produced by pyrolysis of a sample in the lower

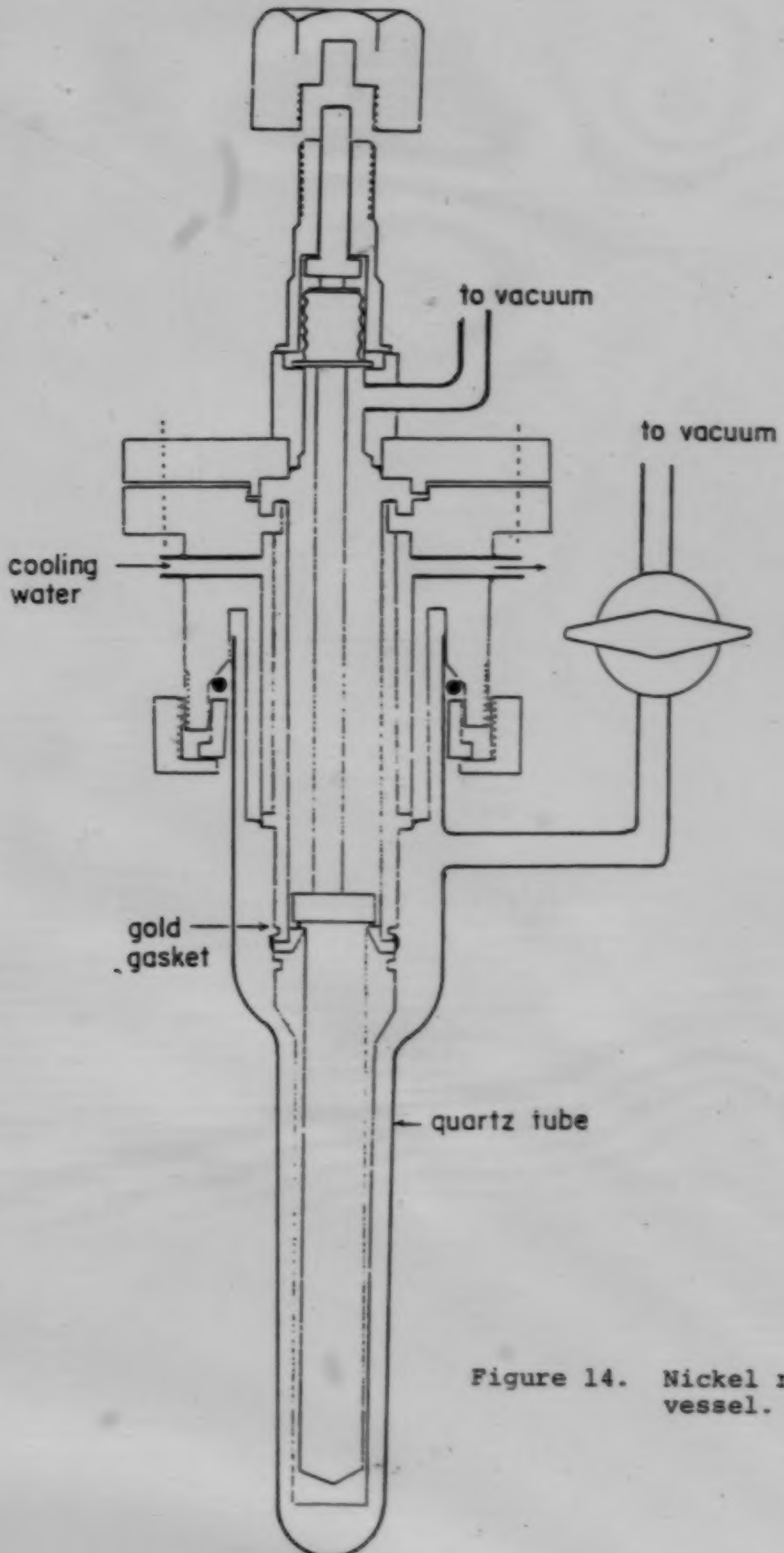

to vacuum
to vacuum
cooling water
gold gasket
quartz tube

Figure 14. Nickel reaction vessel.

combustion chamber. Gases produced in this chamber are contained by a stainless steel plunger which forms a vacuum tight seal with a gold gasket placed on the rim of a stainless steel insert. At the pyrolysis temperature of 1150°C, hydrogen diffuses through the 1.5 mm thick walls of the combustion chamber and is adsorbed on a coconut charcoal trap immersed in liquid nitrogen.

The upper part of the combustion chamber is water-cooled to avoid damage to the teflon vacuum seals. To contain the hydrogen a quartz sleeve surrounds the combustion chamber. A viton O-ring placed between the cooling jacket and the quartz produces an air tight seal when the brass collar is tightened against the brass ring. The addition of a quartz sleeve to the reaction vessel has two distinct advantages.

1. Hydrogen gas diffusing through the vessel wall can be collected quantitatively.
2. Since the nickel reaction vessel is never exposed to air at high temperature, oxidation of the vessel surface does not take place and the vessel has an indefinite life.

However, prolonged heating of the quartz sleeve at high temperature causes gradual devitrification of the quartz and the lower part of the quartz sleeve becomes opaque and starts to deform. This does not appear to affect the performance of the apparatus but the sleeves are replaced after about 30 analyses.

1.2.2. Procedure

1.2.2.1. Organic Material

20 to 30 mg of dry organic sample are weighed into a nickel boat and transferred to the combustion chamber. The gold gasket is positioned and the vessel attached to the vacuum line. It is evacuated and then the quartz sleeve is opened to vacuum. The lower chamber is then sealed off and the quartz sleeve is connected to the charcoal tube. The sample is then pyrolysed at 1150°C for 2 hours. Hydrogen gas is continuously collected on the coconut charcoal trap immersed in liquid nitrogen. During the pyrolysis, two pirani vacuum gauges are used to monitor the hydrogen pressure in the sleeve and any possible gas leak through the gold seal. When combustion is complete the plunger is raised and the oxygen-containing gases are collected on a silica gel trap immersed in liquid nitrogen. Between pyrolyses, the combustion chamber is cleaned with a rotary brush and then with a nylon rotary brush using acetone as solvent and lubricant. The vessel is then flushed with acetone to remove carbon and ashes.

The CO fraction is converted to CO_2 and C in a high voltage discharge maintained between platinum electrodes. The CO is introduced slowly, in aliquots, to the discharge tube and a 4000V potential difference applied using a neon luminous tube transformer. A characteristic blue discharge is produced and CO is converted to CO_2 gas. When the conversion is complete, the CO_2 gas formed is combined with the CO_2 fraction from pyrolysis and the yield measured manometrically. Hydrogen yields are measured using a Toepler pump.

1.2.2.2. Water Analysis

1.2.2.2.1. Quartz Capillary Method

For water analyses, a quartz capillary tube was used to transfer water samples into the combustion chamber. 15 to 20 mg of water are drawn into a quartz capillary which is then sealed and placed in the reaction vessel along with 20 mg of carbon powder which has been degassed by heating at 1150°C for 2 hours. The carbon and capillary are heated to about 500°C for 1 hour to degas the carbon. At this temperature the quartz capillary does not break. The combustion chamber is then sealed and the temperature raised to 1150°C. The capillary explodes and reaction between the H_2O and C starts:

$$2C + 3H_2O \rightarrow CO + CO_2 + 3H_2. \qquad [42]$$

Care should be taken to use excess carbon to promote complete reaction. Insufficient amounts of carbon results in oxidation of the nickel wall, which is difficult to remove. The remainder of the procedure is exactly the same as in pyrolysis of organic material.

1.2.2.2.2. Syringe Method

An alternative method of transferring water into the combustion chamber was tried. After degassing the carbon, dry nitrogen at about atmospheric pressure is introduced into the quartz sleeve. The lower part of the combustion chamber is then immersed in liquid nitrogen. After waiting 10 minutes for the nickel vessel to cool, about 15 μl of water are injected from a micro-syringe through a viton diaphragm and frozen into the combustion chamber. During this operation the remainder of the apparatus is warmed to avoid adsorptive losses of the water. It is essential that the transfer of the water be quantitative to avoid isotopic fractionation. After the water is frozen into the combustion chamber and the chamber sealed, the nitrogen is pumped out of the quartz sleeve and the pyrolysis started.

1.2.3. Result and Discussion

Nickel has been used to purify hydrogen by diffusion (57,64). In general, the rate of diffusion of hydrogen through palladium is greater than through nickel under identical conditions. However, palladium at high temperature (>300°C) quickly develops a coarse crystalline structure and breaks down after some hours of operation. On the other hand, nickel is stable even at 1200°C. Furthermore, continuous use of the nickel reaction vessel has produced no signs of physical damage or any less effective performance.

The rate of diffusion of hydrogen through the nickel wall is a function of temperature, pressure difference, and thickness of wall. For a constant temperature, the rate of diffusion through the wall per unit length is given by

$$q = \text{const.} \times p^{1/2} rd \qquad (r >> d) \qquad [43]$$

where r = radius of tube, d = thickness of nickel wall and p = differential pressure.

Whatman cellulose was pyrolysed in the nickel reaction vessel and the hydrogen which diffused through the nickel wall was collected with a toepler pump. The yield of hydrogen was measured manometrically. The pyrolysis was stopped after 70 minutes when hydrogen ceased to be evolved. Fig. 15 shows yields of hydrogen as a function of time at various temperatures. It is apparent that the rate of diffusion through the wall of the nickel reaction vessel is a function of temperature. The rate of diffusion increases as the temperature increases. Thus, for rapid and complete pyrolysis, a high temperature is desirable. However, the melting point of quartz sets an upper limit to the reaction temperature. The optimum pyrolysis temperature for isotopic analyses was found to be 1150°C. At this temperature, no significant physical deformation of the quartz sleeve was observed. A higher temperature rapidly deteriorates the furnace by oxidizing the heating element.

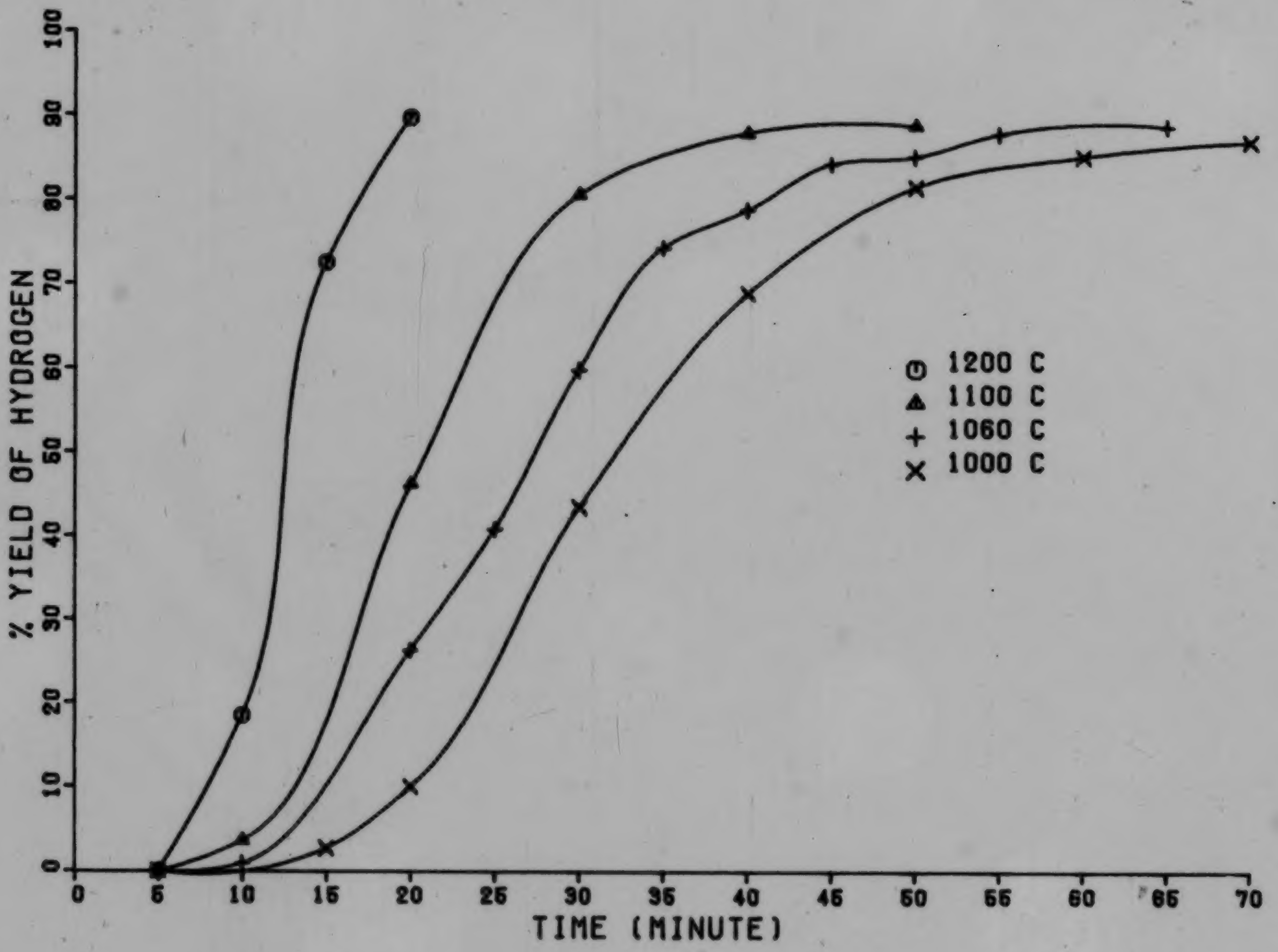


Figure 15. Percentage yield of hydrogen as a function of reaction time for various reaction temperatures.

At 1150°C, pyrolysis and separation of hydrogen by diffusion are complete in 1.5 hours. To prevent possible incomplete hydrogen extraction and isotope fractionation associated with it, most pyrolyses were allowed to proceed for 2 hours at 1150°C. Progress of the reaction was monitored by measuring the residual pressure of hydrogen in the quartz sleeve. Mass spectrometric analysis of the pyrolysis products of cellulose and cellulose nitrate show no measurable amounts of hydrogen or any other hydrogen-containing gases in the vessel, indicating complete pyrolysis. Unlike static pyrolysis in the quartz tube, diffusion of hydrogen through nickel walls forces the reaction to completion.

To test the nickel reaction vessel method, a number of water samples were analyzed and the results were compared with those obtained by conventional methods. The results are shown in Table 8.

Within experimental error the δD and $\delta^{18}O$ values for the standard waters agree with the accepted values. The reproducibility of the method is about ±1°/₀₀ for hydrogen and ±0.2°/₀₀ for oxygen, which is comparable to other methods.

Table 8 Hydrogen and oxygen isotopic compositions of Standard Waters

(A) δD values of Standard Waters (°/₀₀) (SMOW)

Method	Water Sample			
	NBS-1	GISP	SLAP	SHW4[1]
Carbon Reduction				
(i) syringe	-	-189(2)	-418(1)	-
(ii) quartz capillary	-48(8)	-187(2)	-424(2)	-117(4)
Uranium Reduction	-48(2)	-187(2)	-423(4)	-117(2)

(B) $\delta^{18}O$ values of Standard Water (°/₀₀) (SMOW)

Method	Water Sample		
	NBS-1	GISP	SLAP
Carbon Reduction			
syringe	-7.8(2)	-24.5(2)	-54.8(2)
CO_2-H_2O equilibrium	-7.9[2]	-24.7(2)	-55.4(2)

[1] SHW4 is one of the internal working standards

[2] Value taken from Thompson and Gray (96)

Number in parenthesis is the number of analyses.

There are two distinct applications of this carbon reduction method for isotopic analysis of water. One is the simultaneous measurement of δD and $\delta^{18}O$ values of water samples. The other is the measurement of $\delta^{18}O$ values in milligram quantities of water. This method uses 0.015-0.02 ml of water whereas the CO_2-H_2O equilibration method requires relatively large amounts of water (0.5-30 ml) (27,31).

The results of replicate analyses of various organic compounds are shown in Table 9.

While the method is quite suitable for compounds with a low carbon to oxygen ratio in the molecule (C/O), it is less suitable for compounds with a relatively high C/O ratio. For example, the hydrogen yields of citric acid, cellulose nitrate and glycine are 100% whereas those of cellulose and dextrose are lower. The reproducibility of δD measurements on citric acid and cellulose nitrate are excellent (±0.5°/₀₀). On the other hand, reproducibility of δD measurements for cellulose and dextrose are relatively poor.

The low hydrogen yield and poor reproducibility can be related to tar formation during pyrolyses. Compounds with a high C/O ratio tend to form tars during the early stages of pyrolysis which condense onto the relatively cool upper parts of the combustion chamber and are never completely combusted. The problem was partially solved by adding about 50 mg of $ZnCl_2$ to the reaction vessel along with the sample.

Table 9. δD and $\delta^{18}O$ values for some selected organic compounds

Sample	H_2O Yield (%)	δD (°/∘∘NBS-1)	$\delta^{18}O$ (°/∘∘SMOW)	C/O ratio
Cell (5)*	94±1	-37±5	30.1±0.2	6/5
+$ZnCl_2$(8)	96±1	-12±5	30.1±0.2	6/5
dext (17)	96±1	47.4±3.5	27.8±0.4	1
+$ZnCl_2$(14)	99±1	59.3±2.7	28.2±0.4	1
citric(4)	100±1	29.5±0.5	-	6/7
CN(8)	102±1	-5.7±0.5	-	6/11
glycin(8)	100±1	49±3	-	1

*Number in parantheses is number of analysis

Cell = cellulose, dext = dextrose, and citric = citric acid

CN = cellulose nitrate

Lewis acids such as $ZnCl_2$ and $HgCl_2$ have been used extensively in the hydrocracking of coal (111). HCl from the thermal reaction between cellulose and $ZnCl_2$ catalyzes the nonglycosidic condensation and charring reaction of the very early stages of heating (300°C) (62). Further heating of nonglycosidic condensation products leads to carbonization. Thus the role of $ZnCl_2$ is to start the decomposition of cellulose at low temperature and to prevent condensation of volatile products on the upper parts of the reaction vessel.

Addition of $ZnCl_2$ increases both hydrogen yields and δD values, indicating considerable isotopic fractionation during the early stages of pyrolysis. For example, the D/H ratios of cellulose with $ZnCl_2$ present were 26°/oo higher than without $ZnCl_2$ when the hydrogen yield increases by only 2%. Since virtually no oxygen is included in the tars, $\delta^{18}O$ values are unchanged when $ZnCl_2$ is added. This also demonstrates that neither extraneous oxygen nor moisture from the air is introduced with the $ZnCl_2$ even though $ZnCl_2$ absorbs moisture readily.

The use of $ZnCl_2$ however was not entirely satisfactory, particularly with cellulose, as yields never reached 100%. The relatively high standard deviation of replicate analyses reflects the uncertainty of the method.

If hydrogen isotopic analysis alone is desired, tar formation in conjunction with the pyrolysis of compounds of high C/O ratios may be reduced by introducing either a small amount of oxygen gas or other oxidizing agent with the sample into the reaction vessel.

Nitrogen does not appear to interfere with the quantitative extraction of hydrogen or oxygen from organic samples. No attempt was made to separate the CO and CO_2 fractions from nitrogen or nitrogen oxides when the nitrogen containing compounds were analyzed. If nitrogen oxides were formed during the pyrolysis, oxygen isotopic fractionation between CO, CO_2 and nitrogen oxides would be inevitable. However, the mass spectrometric scans on the pyrolysis products of glycine and intronaphthalene showed no traces of nitrogen oxides such as NO or NO_2. This suggests that pyrolysis results in nitrogen being in the molecular form, N_2. This makes it possible to measure the oxygen isotopic composition of the nitrogen-containing compounds.

Table 10 shows the results of test for memory effect.

Table 10. Memory effect of pyrolysis of cellulose nitrate in nickel reaction vessel

Date	Sample	δD (°/₀₀NBS-1)
July 26	Whatman	-6
Aug. 1	Whatman	-5
Aug. 4	Spruce (I)	-118
Aug. 10	Spruce (II)	-128
Aug. 14	Whatman	-5
Aug. 20	Spruce (III)	-90
Aug. 22	Spruce (III)	-91

Alternating pyrolyses of cellulose nitrates of different isotopic composition show no memory effect in the respective δD values. Similar tests were carried out using two cellulose samples differing in D/H ratios by 170°/₀₀. When the vessels were cleaned between analyses with a rotary wire brush, no significant memory effects were observed in spite of the tar formation.

2. Chemical Treatments of Wood Sample

2.1. Extraction of Cellulose

For isotopic analysis, cellulose was isolated from woody tissue according to the standard sodium chlorite method described by Green (53). The procedure begins with the cutting of wood samples to pass 40 mesh in a Wiley mill followed by removal of the extractives with 1:1 benzene and methanol for 24 hours. Treatment with the organic solvents is necessary because the benzene-methanol soluble fractions are so depleted in deuterium that even relatively small amounts of extractives in the sample might alter the final measured D/H ratio (31). As an additional precaution, samples are further washed for 24 hours with acetone. The extractive-free wood samples thus treated are vacuum dried at 40°C.

About 1 gram of the dried sample is suspended in 300 ml of hot water in a 1 litre Erlenmeyer flask containing 3 ml of glacial acetic acid. Then 10 grams of technical grade sodium chlorite is added. The flask is stoppered with an inverted beaker and heated for one hour at 90°C. The whole chloriting operation, including washing, should be done in a well ventilated hood because chlorine dioxide, generated during the reaction, is very toxic. After the chlorite treatment, fresh portions of acetic acid (3 ml) and sodium chlorite (10 g) are added. This step is repeated twice resulting in a total of 3 steps of chloriting treatment. The final residue should be nearly white and retain the woody structure of the original sample. When a satisfactory degree of whiteness has been attained, the solid residue is filtered on a Buchner funnel and thoroughly washed, first with hot water to prevent redeposition of oxidized lignin, and then with large amounts of cold water. This solid residue is often referred to as holocellulose.

2.2. Alkaline Extraction of Holocellulose

Holocellulose thus prepared contains an appreciable amount of non-glucan polysaccharides referred to as hemicellulose. Hemicellulose consists mainly of xylan and manan with small amounts of other polysaccharides, such as galactan, araban and glycurons. The isotopic compositions of hemicellulose may not be the same as those of cellulose due to the difference in chemical structure, molecular mass and chemical bonds in the molecules. To eliminate the possible fractionation associated with inhomogeneity in chemical constitutents of the sample, holocellulose is further treated with sodium hydroxide to give α-cellulose which is the purest form of cellulose one can obtain experimentally.

Holocellulose is transferred to a beaker with 50 ml of 17% NaOH solution. The mixture is stirred at room temperature for 40 minutes. During this treatment, hemicellulose dissolves in the water phase leaving only α-cellulose behind. The mixture is often slightly brown in color because of the presence of some lignin. Later in the alkaline treatment, extracts become colorless.

Holocellulose in general contains up to 2% of klason lignin. The subsequent alkaline extraction removes most of the lignin leaving only traces. This alkaline extraction serves as a means of checking whether delignification with the sodium chlorite technique was successful or not. When the holocellulose, on contact with sodium hydroxide, turns red in color, delignification is not complete. In this case, one more step of chlorite treatment was necessary for the complete removal of lignin.

The mixture is then filtered by suction on a fritted glass filter. α-cellulose thus extracted is washed first with 17% sodium hydroxide to prevent redeposition of lignin and hemicellulose and then with 10% acetic acid to neutralize the sodium hydroxide. The cellulose is then rinsed with a large amount of distilled water. The cellulose is then dried by solvent exchange with acetone on a buchner funnel and further dried in vacuo at 40°C for 2 days. The dried cellulose was then used for oxygen isotopic analysis. For hydrogen isotope measurement, the cellulose is nitrated.

3. Nitration Method

For hydrogen isotope analysis, cellulose and other chemicals are nitrated in order to eliminate the problems associated with exchangeable hydroxyl hydrogen in cellulose.

3.1. Preparation of Nitrating Mixture

The nitrating acid mixture was prepared according to the method of Alexander and Mitchell (2). This involves the slow addition of 404 grams of phosphorus pentoxide to 1000 grams of cold 90% nitric acid to produce an acid mixture of 64% HNO_3-26% H_3PO_4-10% P_2O_5.

1000 grams of 90% fuming nitric acid is transferred to a 2 liter 3 neck-flask. The flask is kept ice cold by immersion in an ice-water bath. 404 grams of phosphorus pentoxides are added to the nitric acid. During the addition of P_2O_5, the acid is swirled continuously with a mechanical stirrer made of a glass rod and teflon wings. Care should be taken not to allow too much atmospheric moisture to get into the acid mixture. 6 or 7 wide mouthed powder funnels are recommended to prevent the pentoxide from sticking to the funnel, absorbing moisture and getting into the acid mixture. With occasional swirling, the solution is complete in a few hours and the acid mixture is then filtered through glass wool into a glass-stoppered bottle and stored in a cool dark place.

Alexander et al (2) also have shown that the age of nitrating mixture has a direct bearing on its nitrating performance. The nitrating performance of the acid mixture was poor before 3 days and after 2 months of age. Thus, the acid mixtures used in this study were aged between 3 days and two months after make-up. Deterioration of the acid was indicated by a marked increase in orange color due to oxides of nitrogen.

3.2. Nitration of Wood

Milled wood is nitrated according to the procedure described by Goring and Timell (47). Approximately 500 mg of extractive-free wood are rapidly immersed in 40 ml of the nitrating acid mixture which has been cooled to -16°C in a ethanol-dry ice slush. Due to the violent reaction particularly between lignin and the nitrating acid, incomplete immersion of wood samples in the acid often results in burning of the sample. The reaction is allowed to proceed in a refrigerator kept at 0 - 5°C for about 24 hours.

The mixture is then cooled to -16°C and filtered through a sintered glass filter. Suction is applied and at the same time the sample is gently pressed with a stainless steel tamp which is roughly the area of the filter. This gentle pressing of the sample with the tamp not only removes the maximum amount of the acid but also minimizes the moist air drawn through the sample. The solid residue is immediately washed with a 1:1 mixture of glacial acetic acid and water cooled to -16°C. Then the sample is immersed in cold water (0°C) and neutralized by adding a small amount of powdered sodium carbonate. The nitrate is washed three times with water. The nitrate is stabilized by three 5-minute treatments with 60 ml each of boiling water. The filtrate is then drained, soaked for 5 minutes in 50 ml of methanol and drained again with suction. The resulting nitrated wood is dried in vacuo at room temperature and is then divided into two aliquots for further analysis.

For the extraction of cellulose nitrate, one aliquot of the nitrated wood is dissolved in 80 ml of acetone with vigorous stirring for about one hour. During this process, acetone dissolves mostly cellulose nitrate with a trace of hemicellulose leaving others precipitated. The viscous mixture is then centrifuged and the clear solution decanted. A large amount of cold water is quickly poured into the solution to give a fibrous precipitate of cellulose nitrate. Cellulose nitrate thus obtained is washed on the glass filter with a large amount of water followed by washing with methanol. The white fibrous cellulose nitrate is further dried in vacuo at room temperature for 2 days.

Unlike nitration of cellulose, cellulose nitrate obtained from the direct nitration of wood is often light brown in color probably due to impurities, such as coloring matter and a trace of nitrated hemicellulose and lignin. No attempt has been made to remove this problem.

3.3. Nitration of Cellulose

The cellulose samples prepared by the sodium chlorite method were nitrated in a manner similar to the direct nitration of wood. 100-200 mg of cellulose is nitrated with 20 ml of the nitrating acid mixture cooled to 0°C in an ice water bath. Unlike wood, addition of cellulose to the acid mixture does not require further cooling with the ethanol-dry ice slush. A reaction time of 3 hours is sufficient for the nitration of cellulose. The rest of the procedure is exactly the same as the procedure employed for the direct nitration of wood.

3.4. Discussion of Nitration

The purpose of nitrating cellulose is to eliminate the exchangeable hydroxyl hydrogen in cellulose enabling the δD value of the non-exchangeable CH hydrogen in cellulose to be measured. The nitration process, however, involves a series of chemical treatments which may introduce secondary isotope fractionation into the system. Therefore it is essential to ensure that the nitration process does not alter the original isotopic composition of CH hydrogen in cellulose.

Epstein et al (31) have shown that the benzene-methanol mixture has, within experimental error, no significant effect on the D/H ratio of the nitrated cellulose.

Two cellulose nitrations with acid mixtures of different isotopic compositions showed no difference in the δD values of the resulting cellulose nitrates, that is, no isotope exchange between the hydrogens in CH group of cellulose and the nitrating acid (31). The effect of acetone on the isotope ratios during cellulose nitrate dissolution was examined by nitrating pure cellulose before and after acetone treatment (see Table 11). The acetone treatment has essentially no effect on the δD values of the samples.

Table 11. δD values of cellulose nitrate before and after undergoing acetone dissolution.

Sample	Nitration δD	+Acetone δD
Cellulose	-54±2 (12)	-53±1 (5)

It is not possible to obtain complete nitration of OH group in cellulose without affecting to some extent the original isotopic composition of the cellulose. A longer nitration time may increase the degree of substitution of OH group but will inevitably degrade the cellulose structure and thereby alter the original isotopic composition of the cellulose. Thus it is essential to find the optimum nitration conditions that give the maximum degree of substitution but minimum degradation of samples.

To find the optimum reaction time, cellulose and samples of wood were nitrated for various lengths of time. The results are shown in Table 12. The duration of nitration depends upon the type of material to be nitrated. The reaction time of one hour appears sufficient to complete the nitration of the exchangeable hydrogen of cellulose.

Similar results have been reported by Timell (98) in which one hour reaction times were found to be sufficient.

Table 12. The effect of duration of nitration on δD values of the resulting cellulose nitrates.

Sample	Duration	δD(°/₀₀)
Whatman -Cellulose	1 hour	-54±2(3)
"	2 hours	-54±1(3)
"	3 hours	-53±1(3)
"	5 hours	-54±1(3)
spruce-1	24 hours	-146
spruce-1	40 hours	-145
spruce-2	24 hours	-140
spruce-2	40 hours	-138
spruce-3	24 hours	-143
spruce-3	40 hours	-140

The direct nitration of woodmills requires a longer reaction time (2,53,97,31), and a time of 24 hours appears sufficient. The same nitration time has been used by Epstein et al (31). They also have shown that repeated nitration after the first 24 hour-long nitration has no measurable effect on δD values of the resulting cellulose nitrate.

Temperature also has an effect on the optimum duration of nitration and degradation of the resulting cellulose nitrate. This is illustrated in Table 13. DP represents degree of polymerization. Higher values of DP correspond to increased degradation, therefore a temperature of 0°C gives slightly less degradation but requires longer reaction time because at low temperature the acid becomes syrupy and is slower to penetrate the sample.

Table 13. Effect of nitration temperature on nitrate degree of polymerization (2).

Temperature °C	DP (k=75)
0	1210
20	1140
40	1045

To ensure the completeness of nitration and minimum degradation of sample, a reaction time of 3 hours and a reaction temperature of 0° to 5°C were adopted for the nitration of cellulose samples. For the direct nitration of wood, a reaction time of 24 hours and a temperature of 0° to 5° were chosen.

The reproducibility of nitration of cellulose was tested. Table 14 shows that nitrating acid mixtures prepared separately produce essentially the same δD values of cellulose.

Table 14. Reproducibility of cellulose nitration for Whatman cellulose

Nitrating Acid	Yield (%)	δD(°/₀₀ NBS-1)
Mixture I (8)*	102	-5.7±0.5
Mixture II (4)	102	-6.4±0.5
Mixture III (2)	102	-4.8±0.1

*Number in parenthesis is the number of analyses.

A hydrogen yield of more than 100% may result from nitrations which have not completely replaced all the hydroxyl hydrogens in cellulose. To check the degree of substitution, the % yield of nitration was calculated according to the equation

$$\% \text{ Yield} = \frac{\text{Wt. of Product}}{\text{Wt. of Cellulose}} \times 54.6 \qquad [44]$$

The % yields of nitration of Whatman cellulose were in the range of 96-100%, which is comparable with the result (96-98%) obtained by Green (52).

There are two different ways of obtaining cellulose nitrate; one is the direct nitration of wood mills followed by acetone dissolution (W-CN) and the other is the nitration of cellulose which has been prepared by the standard sodium chlorite method (W-C-CN). It is important to check whether these two different methods produce cellulose nitrate of the same isotopic compositions. For this, 5 year groups of tree rings of white spruce (WS1) were divided into two aliquots. One aliquot of each group was directly nitrated followed by acetone dissolution (W-CN). The second aliquot was delignified by the sodium chlorite technique including alkaline extraction to produce α-cellulose. The resulting cellulose was then nitrated (W-C-CN). One soft wood and a hard wood were also nitrated in the same ways. The hydrogen isotope results are shown in Table 15.

It is interesting to note that there is a systematic difference in δD values between the two different nitrating methods. Cellulose nitrate prepared by W-C-CN are enriched

Table 15. Isotopic compositions of cellulose nitrates prepared from the direct nitration (W-CN) and the nitration of cellulose prepared by sodium chlorite method (W-C-CN).

Sample	W-C-CN δD(°/oo) yield	W-CN δD(°/oo) yield	ΔδD (°/°°)
WS1-1	-139 (2)	-146 (2)	7
WS1-2	-129 (2)	-139 (2)	10
WS1-3	-134 (2)	-142 (2)	8
WS1-4	-141 (2)	-150 (2)	9
WS1-5	-131 (2)	-137 (2)	6
WS1-6	-132 (2)	-137 (2)	5
WS1-7	-139 (2)	-148 (2)	9
WS1-8	-131 (2)	-139 (2)	8
Soft Wood	-78±1 (6) 100%	-85±1 (5) 101%	7
Hard Wood	-68±1 (6) 100%	-82±3 (6) 104%	14

Number in parenthesis represents number of run.

in deuterium relative to that prepared by W-CN. The average difference is about 7°/oo. The phenomenon is even more obvious for the hard wood where the isotopic difference is 14°/oo.

The lower δD value of cellulose nitrate prepared by the direct nitration of wood may be attributed to the presence of impurities, such as hemicellulose, coloring matter and lignin whose δD values are lower than that of cellulose. As mentioned above, the final products of the direct nitration of wood are slightly brown in color indicating the presence of impurities.

In particular, the presence of hemicellulose especially manan in the cellulose nitrate from the direct nitration may account for this phenomenon. Timell (97) has shown that cellulose nitrate produced by the W-CN method contains small amounts of hemicellulose such as xylan and mannan, and that W-CN contains slightly more mannan than W-C-CN. Xylan, when nitrated, becomes completely insoluble in all solvents and partial solubility in acetone is realized only after the molecular weight has been considerably reduced. On the other hand, nitrated mannan and presumably gluco mannan are easily soluble in acetone. Thus mannan in W-CN, having a low deuterium composition would lower the apparent δD value of cellulose.

Due to previous treatments such as sodium chlorite and alkaline extractions, impurities are largely removed from the cellulose which is further purified during the subsequent nitration followed by acetone dissolution. Thus nitration of α-cellulose shows better reproducibility of δD measurements than the direct nitration of wood followed by acetone dissolution.

Incomplete nitration of wood is also a possible problem. For example, the hard wood shows a rather large difference in δD values between the two different methods. The high yield (104%) of hydrogen for the direct nitration of hard wood indicates incomplete replacement of OH hydrogens in cellulose. This may result from an insufficient amount of the nitrating reagent or insufficient reaction time. Wood requires a longer nitration time because the nitrating reagent must break the woody structure in which lignin and cellulose are interbedded (46). Timell (97) used 120 hours of reaction time for the nitration of the hemlock wood. The optimum conditions for the direct nitration of wood depend upon the type of wood samples.

CHAPTER IV

EXPERIMENTAL RESULTS AND DISCUSSION

1. Identification and Description of Tree Samples

Three of the tree samples chosen (denoted WS1, WS2 and WS3) are white spruce (Picea Glauca) from Edmonton, Alberta. WS1 and WS2 were collected from White Mud Creek, a site having a small stream nearby. WS3 was collected from a flat site with no immediate source of water in the vicinity. White spruce is a natural species in the Edmonton region. WS1 and WS2 are believed to be natural stance while WS3 may have been transplanted at an early age. These tree samples are listed in Table 16. There are also 5 conifers from various growth sites in North America.

2. Hydrogen Isotopic Analysis

To investigate the functional relationship between δD values of tree ring sequences and temperature, δD values of cellulose nitrate in tree rings were determined for the three white spruce samples, WS1, WS2 and WS3. Each tree was divided into five-year groups of growth rings. Thus, the D/H ratio of each group represents the mean D/H ratio of the five consecutive tree rings. The isotopic composition of the five year average is therefore biased toward that of the wider rings. No attempt has been made to normalize the amount of wood contributed by each of the rings to the materials subsequently analyzed.

2.1 Juvenile Rings and Mature Rings

In Fig. 16 the δD values of five year groups of the spruce samples WS1, WS2 and WS3 are plotted against the corresponding growth periods, and compared with mean annual temperatures of Edmonton. The general patterns of δD variations for the three spruce trees are in relatively good agreement with the variation of the mean annual temperature.

One striking feature is that the early growth rings, referred to as juvenile rings, of all three tree samples have lower δD values than the mature growth rings indicating the possible existence of two different isotope scales within a single tree. For example, the juvenile rings of WS1 and WS2 from the periods 1883 to 1903 and 1888 to 1903 respectively have lower δD values than the mature rings by an average of 15°/₀₀ and the juvenile rings of WS3 are more depleted in deuterium than the mature rings by about 30°/₀₀. This phenomenon can be seen more clearly by plotting δD values against temperature. δD values of cellulose nitrates for the three spruce samples are plotted against mean annual temperatures in Figs. 17, 18 and 19. From these figures, it is clear that there exist two different ring groups and that each of them has different isotope scales.

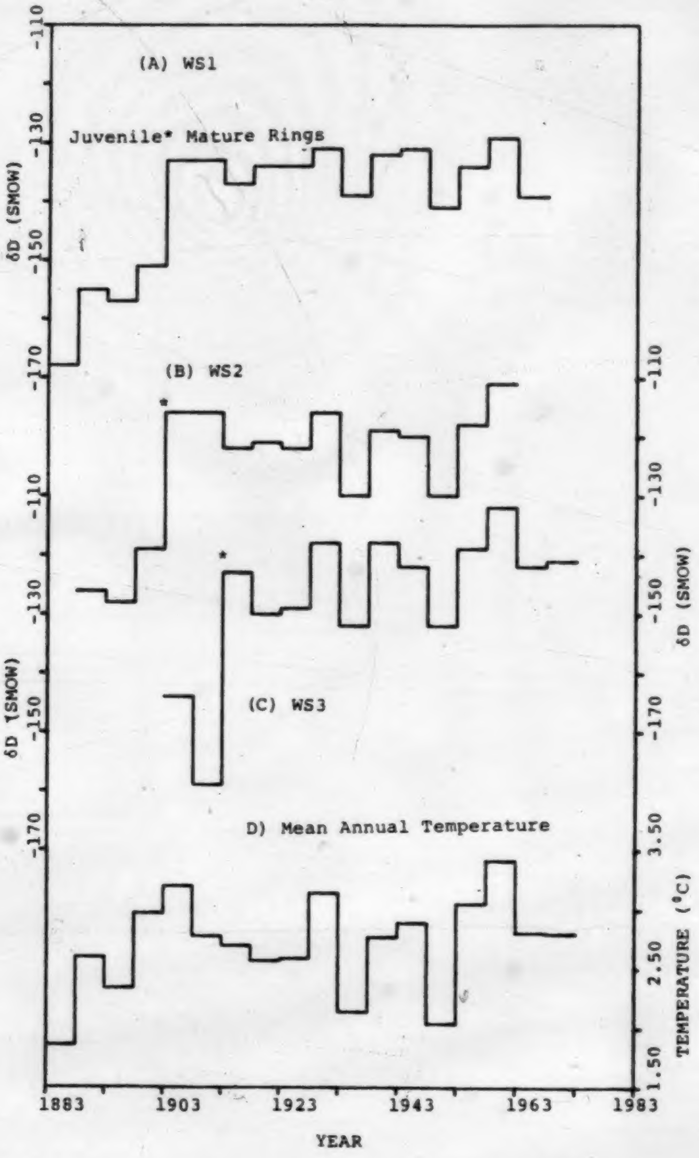


Figure 16. Comparison between δD values of cellulose nitrate and mean annual temperature obtained from the meteorological data A) WS1, B) WS2 and C) WS3. * is the boundary between juvenile and mature rings.

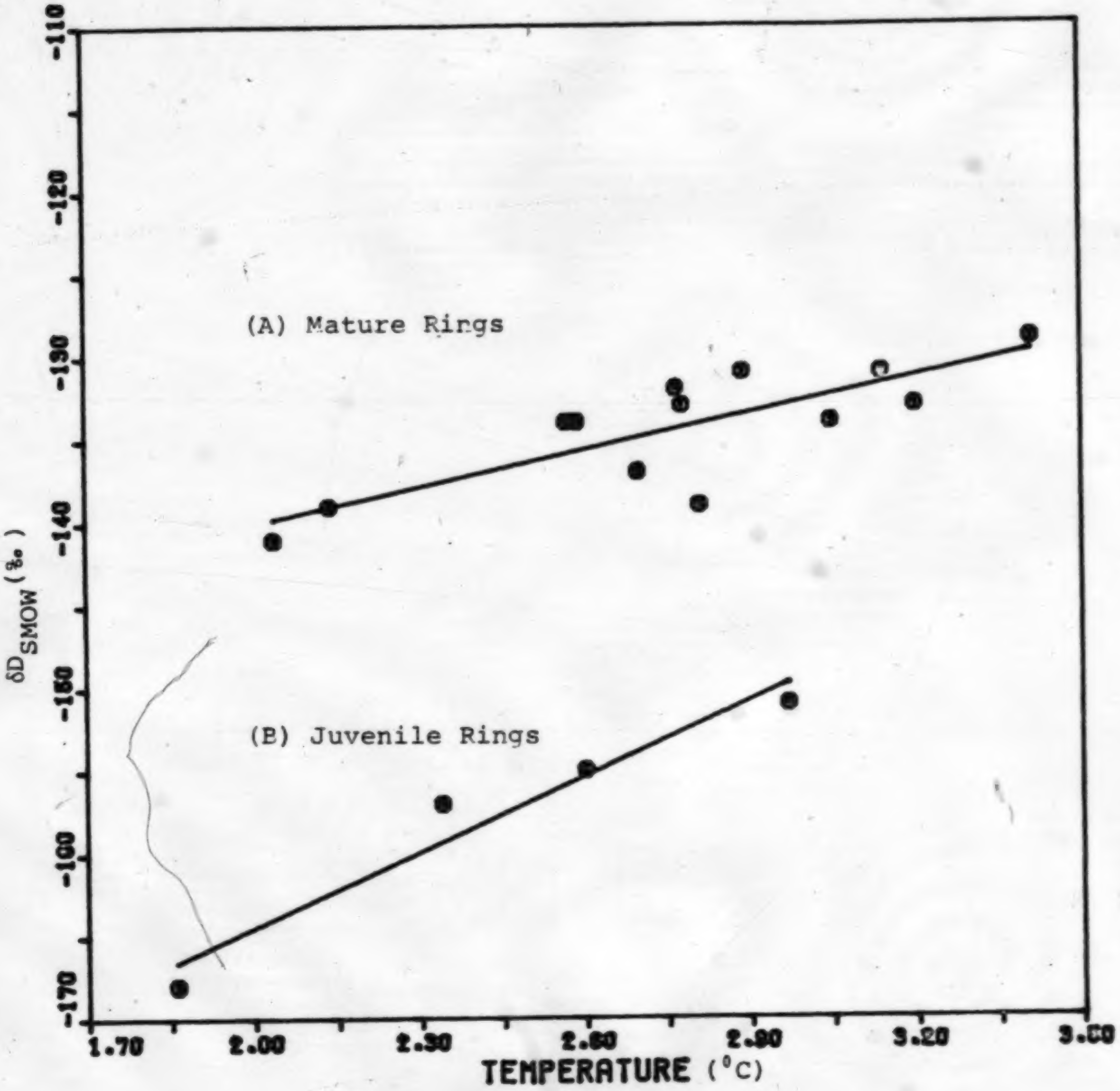


Figure 17. The relationship between δD values of non-exchangeable hydrogen of cellulose (cellulose nitrate) in tree rings of WS1 and mean annual temperature (Sept of previous year to August of growth year). A) Mature rings, B) Juvenile rings.

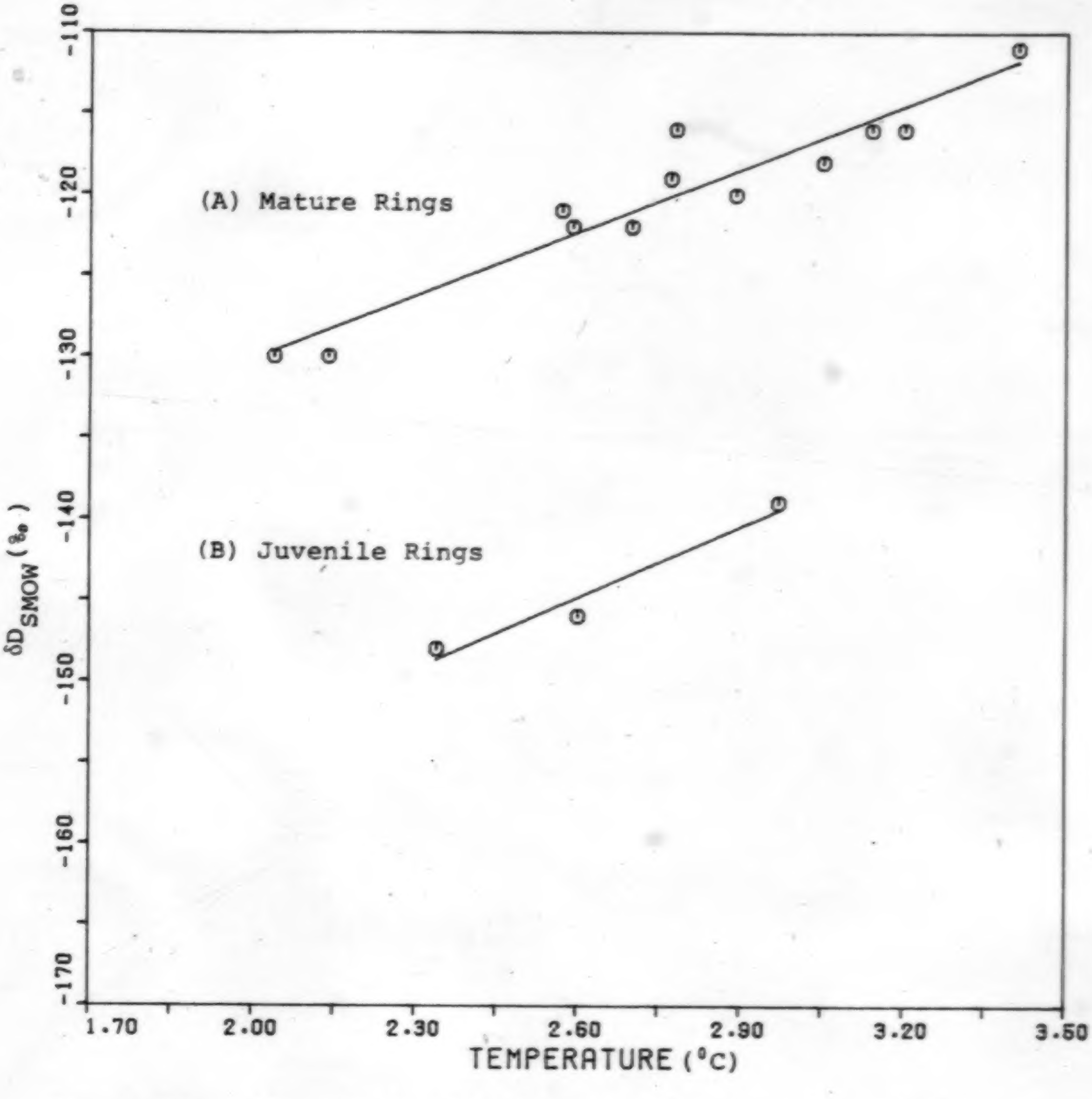


Figure 18. The relationship between δD values of cellulose nitrate in tree rings of WS2 and mean annual temperature (September of previous year to August of growth year). A) Mature rings, B) Juvenile rings.

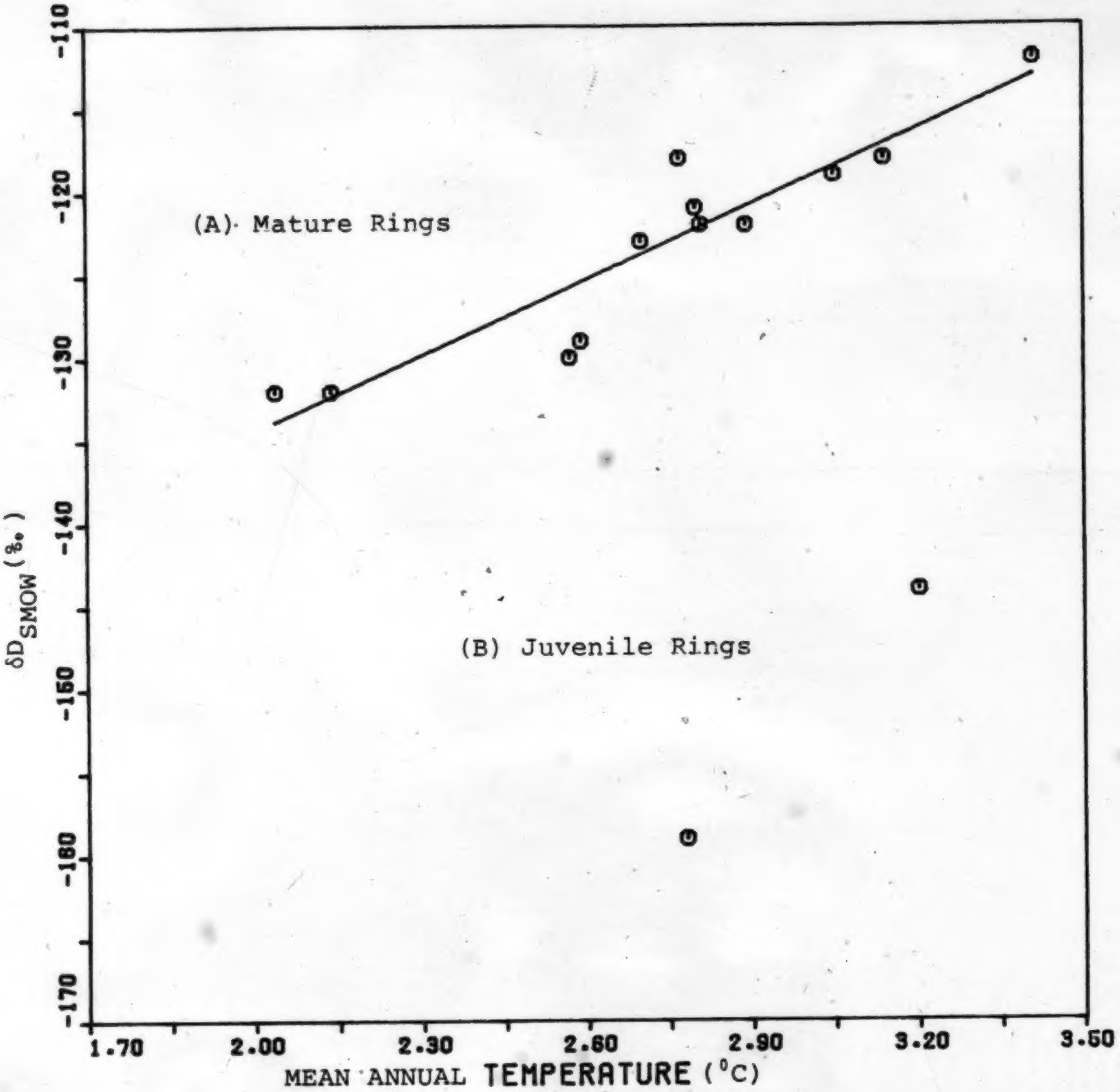


Figure 19. The relationship between δD values in cellulose nitrate of WS3 and mean annual temperature (September of previous year to August of growth year). A) Mature rings, B) Juvenile rings.

Table 16. Identification and Description of Wood Samples

Sample	Growth Period	Location
WS1 (white spruce)	1885-1965	White Mud Creek, Edmonton
WS2 (white spruce)	1890-1965	White Mud Creek, Edmonton
WS3 (white spruce)	1905-1975	Mill Woods, Edmonton
RW1 (redwood)	1890-1974	Grant Grove, California
YSP1 (white spruce)	1915-1975	Decoeli Mt., Yukon
YSP2 (white spruce)	1890-1975	New Kluane Lake, Yukon
FVS (white spruce)	1918-1975	Fort Vermillion, Alberta
VC (cedar)	1918-1968	Vancouver Island, B.C.

The two groups of isotope data cannot be regarded as random scattering of isotope data for a number of reasons.

1. Each group consists of the consecutive growth rings.
2. The difference in δD values between the juvenile and mature rings are in the same order of magnitude as the overall δD variations shown by mature rings and therefore cannot be taken as scattering of data.
3. All three spruce samples from Edmonton show the same phenomenon regardless of their age.

This large difference in δD values between two ring groups does not coincide with any climatic event happening at a particular time. For example, the low δD values of WS3 for the period 1903-1913 are not reflected in the δD values of WS1 and WS2 for the equivalent time period. There was no record of unusual or sudden change in climate between these periods of time. The answer may be found from the physiological factors controlling growth of trees.

During the life of the tree there are normal variations in the structure and the chemical composition of a cell wall, which are associated with the development from the juvenile stage to that of maturity (15). For example, the average cell length increases through the successive growth rings from the center of the tree outward until a more or less constant value is reached. There are also changes in chemical composition as the tree grows. It has been shown that the amount of cellulose increases through the juvenile period (101). All these changes are such that as the age of the tree increases the wood formed becomes more uniform and shows a greater degree of stability throughout the period of maturity than that observed in the juvenile stage (15). Due to the isotopic competition between chemical constituents in wood, changes in structure and relative amounts of chemicals will inevitably alter their isotopic compositions. Thus, such differences between juvenile and mature sections of tree rings may give rise to the large difference in δD values observed. Dendroclimatology encounters a similar problem associated with juvenile rings which are often eliminated from the analysis because they provide the least reliable climatic information (44).

To check whether this large difference in δD values is truly related to changes in physiological properties of plants, growth ring widths of the three spruce samples are plotted against the growth periods in Fig. 20. The ring width in Fig. 20 is an arbitrary unit which shows the relative size of the ring width. The data were smoothed with an 11 year running average which was chosen arbitrarily to illustrate general growth trends. Juvenile rings in dendrochronology are the first 10 to 30 year portion of growth rings which often show increase in ring width. For the three spruce samples, the juvenile sections of tree rings are well matched with the time periods which show the lower δD values of cellulose nitrate. Thus the sudden drop in δD values of cellulose nitrate in early growth rings appears to be a unique characteristic of juvenile rings of white spruce. Thus, ring width data can be a useful tool to differentiate the juvenile rings from the mature. No attempt has been made to find whether other species have the same phenomenon associated with juvenile rings.

In the functional analysis with climatic data, there is no doubt that these two ring groups representing different isotopic scales should either be treated separately or the juvenile ring data be discarded completely. In

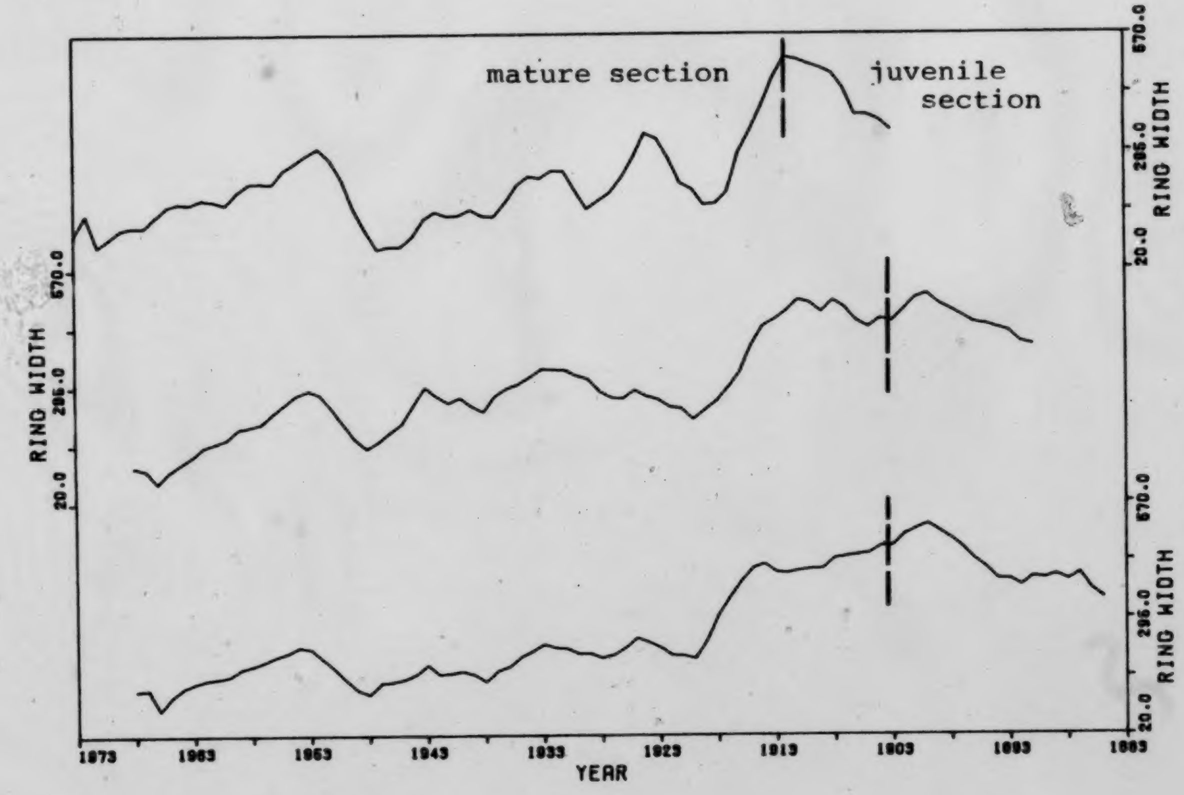


Figure 20. Ring width variations in three spruce samples from Edmonton. Ring width data were smoothed arbitrarily with 11-year running average. The vertical dashed lines represent the boundary between juvenile and mature ring sections.

this study the isotopic analysis of juvenile and mature rings were done separately.

Examples of this sudden drop in δD values associated with juvenile rings may be found in the literature. For example, Yapp et al (108) measured δD and $\delta^{13}C$ values of a branch of a tree from Two Creek Forest in Wisconsin. Most of the δD values varied around a value of -125°/₀₀. However, the oldest 5-year group sample had a δD value 18°/₀₀ lower than this. This was not substantiated by the $\delta^{13}C$ data. They attributed the low δD value of the oldest sample to utilization by the tree of ambient precipitation, rather than the water from Lake Chicago, due to the low level of glacial Lake Chicago during the Valders advance. However, the low δD value may just be the unique property of juvenile rings mentioned above.

2.2 Relationship between Temperature and δD Values of Cellulose Nitrate

The choice of growing period over which the temperatures are averaged is rather subjective. Most trees have a growth period limited to several months rather than the whole year. Thus, the functional analysis of isotope data with temperature involves the selection of the proper temperature group in a statistically meaningful manner. For this purpose, linear regression analysis with δD values of cellulose nitrates and various growing season temperatures was carried out.

Table 17 shows the correlation coefficients between δD values of cellulose nitrate and various mean monthly temperatures. Proper interpretation of the correlation coefficient should include a confidence level which provides information about the probability of the relationship found being pure coincidence. The confidence level of the correlation coefficient is a function of the number of datum points; the more the number of datum points, the higher the level of confidence for a given correlation coefficient. The 95% confidence level is widely used to specify the significance of the functional relationship sought (109). The correlation coefficient equivalent to 95% confidence level in this analysis is 0.58. In other words, there is a 5% chance that the functional relationship found is purely coincidence. Any correlation coefficient greater than 0.58 may be regarded as showing a significant correlation. Despite the fact that trees in Edmonton, which is a relatively dry region, grow in spring and early summer, it is apparent that the monthly temperature of December and January of both the previous and the two previous years show the best correlation with δD values of the tree rings. The monthly temperatures of September, October and November are poorly correlated with δD values of cellulose nitrates for all three spruce samples. This suggests that winter temperature plays an important role in determining D/H ratios of cellulose produced in the subsequent growing season.

Table 17. % Correlation Coefficients of the Linear Regression Function between δD values of Cellulose Nitrate and Mean Monthly Temperature

Sample	Year	J	F	M	A	M	J	J	A	S	O	N	D
WS1	n	88	23	31	49	20	38	5	19				
	n-1	67	10	24	18	48	20	9	12	25	4	13	48
	n-2									24	10	7	79
WS2	n	67	45	30	58	26	52	0.5	58				
	n-1	39	51	22	19	56	30	16	42	2	15	8	55
	n-2									6	43	5	89
WS3	n	53	33	5	56	3	40	11	71				
	n-1	31	44	35	16	33	35	38	69	7	35	13	36
	n-2									19	34	16	68

Note: n = Contemporary year
n-1 = Previous year
n-2 = Second previous year

The importance of winter temperature is also seen in the correlation with seasonal temperature. Table 18 shows the correlation between δD values of cellulose nitrate and various seasonal temperatures. Winter temperature is defined as the average temperature calculated from December of the previous year to March of the contemporary year. Summer temperature is defined as the average temperature of April, May, June, July, and August, which represents the growing season temperature. The samples WS1 and WS2 show

Table 18. Correlation between δD Values of Cellulose Nitrate and Seasonal Temperatures (δD=at+b)

Sample	5-year temperature group	Slope °/oo/°C	Intercept °/oo	Correlation coefficient
WS1	Winter (Dec-Mar)[1]	2.7±0.4	-107±4	0.89
	Summer (Apr-Aug)[2]	4.4±2.0	-189±30	0.47
WS2	Winter (Dec-Mar)	4.6±0.6	-74±6	0.92
	Summer (Apr-Aug)	9.0±3.0	-231±42	0.64
WS3	Winter (Dec-Mar)	3.6±1.0	-87±14	0.65
	Summer (Apr-Aug)	10±3	-250±36	0.74

[1] Winter (Dec-Mar) = Average winter temperature calculated from December of previous year to March of contemporary year

[2] Summer (Apr-Aug) = Average summer temperature calculated from April to August of contemporary year

excellent correlation with winter temperature in contrast to the rather poor correlation with summer temperature. On the other hand, WS3 shows a slightly better correlation with summer temperature than with winter temperature. Nevertheless, correlation of δD values of WS3 with winter temperature is significant and is comparable with the correlation between δD values and summer temperature shown by WS1 and WS2. Most conifers in the Edmonton region grow in spring and early summer, so that it might be expected that δD values of tree rings would be better correlated with this growing season temperature. However, excellent correlation with winter temperature and the poor correlation with seasonal temperatures shown by the three spruce trees indicates that the direct effect of the growing season temperature on the hydrogen isotopic fractionation during photosynthesis is not the major factor in determining the D/H ratios of tree rings. It appears that D/H ratios in cellulose of tree rings reflect that of ground water taken by the plants. The isotopic composition of soil water taken by plants in turn may depend upon the mixing ratio of winter and summer precipitation. For example, the high correlation between δD values of cellulose nitrate and winter temperature shown by WS1 and WS2 may be attributed to the importance of winter precipitation in forming the soil water taken by the trees.

It is unlikely that photosynthesis leading to production of cellulose is vigorous during the winter season especially December and January. However, it is possible that winter precipitation may play an important role in the production of cellulose during the growing season. This may be the case for WS1 and WS2 which came from Whitemud Creek where a natural drainage system is well developed. Thus the summer rain at the time of precipitation drains out to a nearby stream whereas winter snow, due to poor mobility, stays and thereby has a better chance of penetrating the ground for a long period of time. Thus the soil water taken by these plants may consist mainly of winter precipitation. Consequently, cellulose produced by trees utilizing this soil water will reflect mainly the isotopic composition of winter precipitation. On the other hand, for WS3 whose growth site was flat, the contribution of summer precipitation to the δD values of cellulose might be compatible with that of winter precipitation. Therefore it is the isotopic composition of soil water which plays the most important role in determining D/H ratio of cellulose in tree rings.

However, summer temperatures prevalent during the growth year do show reasonable correlation with δD values of tree rings. This may be partly due to the effect of summer precipitation, although small, on the D/H ratio of soil water. The existence of factors other than ground water affecting D/H ratio of cellulose is not completely ruled out. For example, temperature-dependent relative humidity in the growing season may affect D/H ratios of both ground and leaf waters through evapotranspiration, and thereby D/H ratios of tree rings. Unfortunately, lack of relative humidity records excludes rigorous functional analysis between relative humidity and δD values of tree rings.

Various mean annual temperatures were correlated with δD values of cellulose nitrate in tree rings (see Table 19). The mean annual temperature averaged from September of the previous year to August of the growing year shows a better linear correlation with δD values of

Table 19. Correlation between δD values of cellulose nitrate and various mean annual temperatures (δD=at+b)

Sample	5-year temperature group	Slope °/oo/°C	Intercept °/oo	Correlation coefficient (r)
WS1	Previous year (Sep-Aug)[1]	7.3±2	-155±5	0.80
	Contemporary year (Sep-Aug)[2]	5.7±1.3	-150±4	0.79
	Contemporary year (Jan-Dec)[3]	0.6±2	-153±5	0.71
WS2	Previous year (Sep-Aug)	13±1	-156±4	0.95
	Contemporary year (Sep-Aug)	10.3±1.3	-149±4	0.93
	Contemporary year (Jan-Dec)	12±2	-153±6	0.86
WS3	Previous year (Sep-Aug)	15±2	-165±8	0.93
	Contemporary year (Sep-Aug)	11±4	-154±5	0.88
	Contemporary year (Jan-Dec)	14±3	-161±8	0.83

[1] Previous year (Sep-Aug) represents mean annual temperature measured from September of the n-2 year to August of the n-1 year.

[2] Contemporary year (Sep-Aug) represents mean annual temperature measured from September of the n-1 year to August of the contemporary year (n).

[3] Contemporary year (Jan-Dec) represents the mean annual temperature measured from January to December of the contemporary year (n).

cellulose nitrate than the January-December mean annual temperature for all three spruce samples. Taking into consideration that conifers in the Edmonton region grow from April to August and that December-March winter temperatures showed excellent correlation with δD values of cellulose, the mean temperature of September-August is thought to be a reasonable choice for mean annual temperature.

It is of interest to note that mean annual temperature averaged from September of the n-2 year to August of the n-1 year has the best correlation with δD values of cellulose. In other words, the mean annual temperature of the previous year plays a more important role in determining D/H ratios of cellulose in tree rings than the mean annual temperature of the contemporary year. This suggests that the plants analyzed here utilized meteoric water stored in the ground and that the time lag between the actual precipitation and fixation by the plants is about 1 year. Thus the proper grouping of the mean annual temperature that shows best correlation with δD values of tree rings may depend upon environmental factors such as continental or marine environment and other factors such as latitude, altitude, dry or wet climate etc. The fact that the δD correlation with mean annual temperature of the previous year is better than that with the contemporary year may be an artifact caused by the use of 5-year groups of rings. However, the fact that all three spruce samples show the same trend makes this possibility less likely. This will be further discussed with oxygen isotope data.

The linear regression equations describing the relationship between δD values of cellulose nitrate and mean annual temperature are

$$\delta D = (7.3\pm2)t - (155\pm5)°/_{\circ\circ} \text{ for WS1} \qquad [45]$$

$$\delta D = (13\pm1)t - (156\pm4)°/_{\circ\circ} \text{ for WS2} \qquad [46]$$

$$\delta D = (15\pm2)t - (165\pm8)°/_{\circ\circ} \text{ for WS3.} \qquad [47]$$

Both the slopes and the intercepts of these equations are different. In addition, the absolute δD values of the three spruce samples for the time equivalent ring groups are not identical. This may introduce serious problems to paleoclimatic reconstruction especially when two trees of different growth periods are used. Despite the fact that WS1 and WS2 are from essentially the same site, the average δD values of WS1 are lower than those of WS2 by 14°/₀₀ whereas WS2 and WS3 have essentially the same δD values. These may partly be attributed to the difference in the D/H ratio of the soil water taken by the tree samples. The mixing ratio of summer to winter precipitation in the formation of soil water may depend upon factors such as lithology, permeability of the soil and topography of the growth site. Due to differences in such factors, the relative amount of winter precipitation in the soil water taken by WS1 might have been higher than that taken by WS2. Thus the average δD values of WS1 would be lower than that of WS2 and the temperature coefficients different. In support of this, it is interesting to recall the δD values of WS1 were better correlated (0.89) with winter temperature than with mean annual temperature (0.80) whereas WS2 shows better correlation (0.95) with mean annual temperature than with winter temperature (0.92). Since they reflect two different temperatures, both the slope and the intercept are also different.

The number of 5-year ring groups in juvenile sections of WS1, WS2 and WS3 are 4, 3 and 2 respectively. Thus, the number of ring groups may be too small for any meaningful functional analysis between δD values of juvenile rings and climatic parameters. However, they appear to exhibit some important trends that are useful in interpreting isotopic ratios in tree rings. Table 20 shows the correlations between δD values of juvenile rings and various temperature groups. Seasonally, δD values of juvenile rings have better correlation with winter temperatures than with summer temperatures. This is in keeping with the result obtained from mature rings, namely, that trees utilize mainly winter precipitation stored in the ground.

WS1 and WS2 show that δD values of juvenile rings are better correlated with the mean annual temperature of the contemporary year than with the mean annual temperature of the previous year. This is in contrast to the result obtained from mature rings. The result may indi-

Table 20. Linear correlations between δD values in cellulose nitrate of juvenile rings and various temperatures

5-yr temperature group	Slope	Intercept	r
WS1			
Annual (previous)	6.6±1.9	-173±5	0.92
Annual (contemporary)	15±2.6	-195±6.5	0.97
Winter (Dec-Mar)	6.0±1.6	-945±17	0.93
Summer (Apr-Aug)	20±12	-402±143	0.77
WS2			
Annual (previous)	2.0±12	-150±34	0.16
Annual (contemporary)	15±3	-183±8	0.98
Winter (Dec-Mar)	5.4±2.1	-91±21	0.93
Summer (Apr-Aug)	-18±29	+73.7±355	-0.52

Annual (previous) represents mean annual temperature measured from September of the n-2 year to August of the n-1 year.

Annual (contemporary) represents mean annual temperature measured from September of n-1 year to August of the contemporary year (n).

Winter (Dec-Mar) represents winter temperature measured from December of the n-1 year to March of the contemporary year (n).

Summer (Apr-Aug) represents summer temperature measured from April to August of the contemporary year (n).

cate that there are compositional and chronological differences between soil moistures taken by juvenile rings and mature rings. For example, they utilize two different soil moistures whose isotopic composition and time of precipitation are not identical. When trees are young, the roots are shallow and use soil moisture from relatively shallow strata. The shallow soil moisture in turn may be composed mainly of contemporary winter precipitation. On the other hand, the fully developed roots of mature trees absorb the soil moisture stored in deeper strata in addition to shallow soil moisture. The soil moisture in deeper strata may be composed of precipitation from previous years. Then, it is expected that juvenile and mature rings within a single tree may contain two different isotope scales that represent two different mean annual temperatures. However, the difference in the source of soil moisture cannot account for the large difference in δD values between juvenile and mature rings because the annual variation in δD value of water is relatively small. Since the number of juvenile rings is few, compared to mature rings, the interpretation of δD values of juvenile rings may be fortuitous.

As a result of this experiment, it is apparent that there exists an empirical relationship between δD values of non-exchangeable CH hydrogen in cellulose and mean annual temperature and that it is a positive linear relationship with reasonable correlation coefficient.

2.3 Relationship between D/H Ratios of Tree Rings and the Amount of Precipitation

The possibility of a functional relationship between δD values in cellulose nitrate of tree rings and the amount of precipitation was examined. Correlation coefficients of linear regression analysis between them were calculated. The grouping of average amounts of precipitation was done in a similar manner to that of temperature. Table 21 shows the correlation between the amount of monthly precipitation and δD values of cellulose nitrate. The amount of precipitation in the month of January of both previous and contemporary years shows the best correlation with δD values of cellulose nitrate for all three spruce samples from Edmonton. Unlike temperature, the precipitation of December is poorly correlated with δD values of tree rings.

Table 22 shows the linear correlation between δD values of tree rings and the seasonal and annual precipitation. Compared to the correlation with temperature, the D/H ratios of tree rings are poorly correlated with the amount of precipitation in all cases. For example, the best correlation coefficient obtained between winter precipitation and δD values of WS2 is only 0.68 whereas its correlation coefficient with winter temperature is 0.92. Nevertheless, the result of linear correlation between δD values of tree rings and the amount of precipitation is qualitatively in agreement with the result from the temperature-δD relationship.

For all three samples, WS1, WS2 and WS3, winter precipitation averaged from December to March shows a

Table 21. % Correlation Coefficients of the Linear Relationship between δD Values of Cellulose Nitrate and Monthly Precipitation Amount.

Sample	Year	J	F	M	A	M	J	J	A	S	O	N	D
WS1	n	61	28	4	7	28	19	30	50				
	n-1	72	50	13	13	10	14	43	43	22	55	1	10
	n-2									20	47	2	10
WS2	n	60	47	16	32	42	24	41	20	42			
	n-1	69	24	52	24	35	16	48	7	23	38	19	13
	n-2									16	24	13	30
WS3	n	49	1	23	11	37	5	35	22	33			
	n-1	65	40	18	2	39	25	48	3	37	42	8	11
	n-2									22	23	7	18

n = Contemporary year

n-1 = Previous year

n-2 = Second previous year

Table 22. Correlation between δD Values of Cellulose Nitrate and Seasonal Precipitation ($\delta D = aP_r + b$)

Sample	Season	Slope	Intercept	r
WS1	Winter (Dec-Mar)	-1.5±0.9	-122±8	-0.43
	Summer (Apr-Aug)	-0.4±0.4	-129±8	-0.32
	Annual (previous)[1]	-1±1	-120±6	-0.26
	Annual (contemporary)[2]	-1.3±1	-115±15	-0.35
WS2	Winter (Dec-Mar)	-3.4±1	-92±1	-0.68
	Summer (Apr-May)	-0.75±0.1	-108±13	-0.27
	Annual (previous)	-2.9±1.6	-77±25	-0.49
	Annual (contemporary)	-2.6±1.6	-81±23	-0.46
WS3	Winter (Dec-Mar)	-0.7±0.7	-117±6	-0.3
	Summer (Apr-Aug)	-0.2±0.8	-119±6	-0.23
	Annual (previous)	-1.7±1.9	-97±28	-0.29
	Annual (contemporary)	-2.4±1.9	-87±29	-0.36

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[1] Annual (previous) represents average amount of precipitation measured from September of n-2 year to August of n-1 year.

[2] Annual (contemporary) represents average amount of precipitation measured from September of n-1 year to August of contemporary year (n).

better correlation with δD values of tree rings than summer precipitation does. This indicates the importance of winter precipitation in determining δD values of tree rings.

Unlike temperature, winter precipitation shows a slightly higher correlation with δD values of tree rings than does mean annual precipitation. This may suggest that factors other than the δD value of precipitation also play some role in determining δD values of tree rings. In contrast to correlation with temperature, WS1 and WS3 show slightly higher correlation with the mean annual temperature of the contemporary year than with that of the previous year.

In all cases, δD values of tree rings are negatively correlated with the amount of precipitation. The negative correlation is due to the amount effect of meteoric water on its isotopic composition. Dansgaard (16) has reported several possible contributing factors for the amount effect:

1. When a given mass of condensing vapor is considered, the isotopic composition of newly formed condensates becomes progressively depleted in heavy isotopes (D and ^{18}O) as the cooling proceeds. Since the amount of condensate increases with the degree of cooling, the amount effect with a negative correlation between isotopic compositions of water and the amount of precipitation may be expected.
2. Fractionation may occur due to isotopic exchange between the falling drops of condensate and the

environmental vapor. This effect accounts for the relatively high δ values of light rain. In such case, vapor below the cloud has not yet been exposed to cooling processes and thereby retains high values. Thus the isotopic exchange with such vapor of high δ values results in an enrichment of light rain in heavy isotopes (D and ^{18}O). In the case of heavy rain, vapor composition is determined mainly by the liquid phase.

3. Fractionation by evaporation of falling drops. Low humidity of the low altitude air causes a loss of liquid phase by evaporation and thereby considerable enrichment of the rain.

The amount effect is found all the year round at most tropical stations and in the summertime at mid latitude, but never at polar stations (16). The negative correlation encountered for three spruce samples from Edmonton appears to be the result of the amount effects particularly that of fractionation by high evaporation in the Edmonton region. The high evaporation in turn is related to low humidity in this region.

Table 23 shows the correlation between δD values of juvenile rings and the amount of precipitation. Mean annual precipitation of the contemporary year shows a better correlation with δD values of juvenile rings than the mean annual precipitation of the previous year, which

Table 23. Correlation between δD Values of Juvenile Rings and Precipitation

Sample	Season	Slope	Intercept	r
WS1	Annual (previous)	1.4±1.1	-17.8±18	0.65
	Annual (contemporary)	1.7±0.9	-183±14	0.81
	Winter	2±1.3	-174±11	0.73
	Summer	1.1±0.7	-185±16	0.78
WS2	Annual (previous)	1.2±0.02	-162±0.3	1.0
	Annual (contemporary)	1.3±0.1	-166±2	0.996
	Winter	1.6±0.4	-158±4	0.96
	Summer	0.9±0.2	-167±4	0.99

Annual (previous) represents amount of precipitation measured from September of the n-2 year to August of the n-1 year.

Annual (contemporary) represents amount of precipitation measured from September of the n-1 year to August of the contemporary year (n).

is in agreement with the result from temperature data. In contrast to temperature, summer precipitation shows a slightly higher correlation than winter precipitation. The most striking feature is the positive linear relationship between δD values of juvenile rings and the amount of precipitation whereas the mature rings have a negative correlation which is in turn attributed to the amount effect of precipitation. The causes of the positive slope and higher correlation with summer precipitation which is not substantiated by temperature data are not known. They could be an artifact of correlation with a small number of data.

Due to poor correlation between δD values of tree rings and precipitation, it is unlikely that one can obtain any reliable information about the amount of precipitation from D/H ratios of tree rings. However, correlation between δD values of tree rings and precipitation supports the results obtained from correlation with temperature, i.e. D/H ratios of tree rings mainly reflect D/H ratio of soil water taken by the plant.

2.4 Hydrogen Isotopic Analysis of Materials other than Cellulose in Tree Rings

Woods are extremely complex chemical systems in which each chemical constituent has a different isotopic composition. Thus it is necessary to find a material that best preserves climatic information. Alternatives to the use of cellulose may be extractive-free wood, hemicellulose and lignin. Wood extractives are excluded from the isotopic analysis for a number of reasons. Wood extractives include a wide range of chemical types and a very large number of individual compounds. Some of the major chemical types of extractives are 1) terpenes and related compounds, 2) fatty acids, 3) aromatic compounts, and 4) volatile oils (90). The relative amount of wood extractives is so small that isotopic determination of them is almost impossible. In addition, some extractives found in a given growth ring are deposited at different times from the rest of the constituents and thereby destroy the time scale.

To find a functional relationship between extractive free wood and temperature, D/H ratios of extractive-free wood of WS1 were determined. To eliminate the problem associated with exchangeable OH hydrogen, extractive-free wood mills were nitrated. Table 24 compares the δD values of nitrated extractive-free wood with those of nitrated cellulose (see Fig. 21). Extractive-free wood (denoted by wood in the subsequent discussion) has lower δD values than cellulose nitrate by approximately 19°/₀₀. Total variation in δD values of nitrated wood is 7°/₀₀ whereas that of cellulose nitrate is 10°/₀₀. It is of interest to note that the nitrated wood of juvenile ring (#17) also has a low δD value. From Fig. 21, it is apparent that there is no functional relationship between δD of wood and δD values of cellulose.

Table 25 shows correlations between various δD values of chemical constituents in wood and mean annual temperature. Whereas δD values of cellulose nitrates are well correlated with mean annual temperature (r=0.914), δD values of nitrated extractive-free woods are not correlated with the temperature. The extremely low value of the correlation coefficient (r=0.045) suggests that the hydrogen isotopic composition of extractive-free woods does not preserve temperature information. Furthermore, it is to be expected that δD values of whole wood

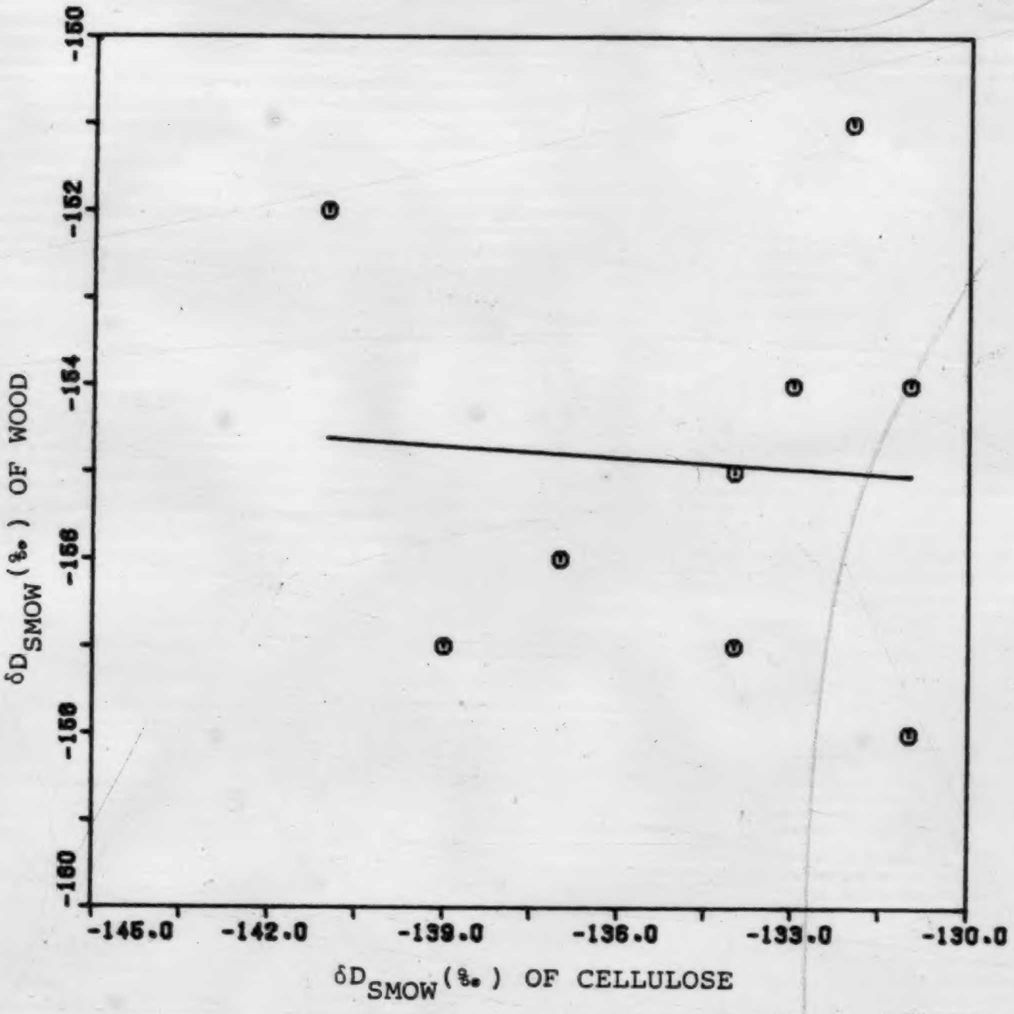

Figure 21. The relationship between the δD values of extractive-free wood from WS1 and the corresponding cellulose. Both wood and cellulose are nitrated to eliminate exchangeable OH hydrogen.

Table 24. Comparison between D/H ratios of Nitrated Cellulose and Nitrated Extractive-Free Wood of WS1

Ring	δD CN* °/oo	δD NW** °/oo	δD Lignin °/oo	ΔδD ($\delta D_{CN}-\delta D_{lig}$) °/oo
4	-141	-152	-174	33
5	-131	-158	-212	81
6	-132	-151	-189	57
7	-139	-157	-193	54
8	-131	-154	-200	69
9	-134	-155	-197	63
10	-134	-157	-203	69
11	-137	-156	-194	57
12	-133	-154	-196	63
17†	-168	-176	-192	24

* CN = cellulose nitrate

** NW = nitrated wood

† No. 17 is a juvenile ring group

Table 25. Relationships between δD Values of Various Wood Components and Temperature

Variable Par	Regression Coefficient °/₀₀/°C	Intercept °/₀₀	Correlation Coefficient (r)
δD_{CN} vs t	9.4±1.6	-159±4	0.91
δD_{NW} vs t	-0.3±2.6	-154±7	-0.045
δD_{NW} vs D_{CN}	-0.042±0.25	-161±34	-0.064
δD_{lig} vs t	-19.7±8.5	-144±22	-0.66
$\Delta\delta D$ vs t	29±9	-156±25	0.76

CN = cellulose nitrate
NW = nitrated wood
lig = lignin

including extractives would not have sensitive temperature information. Thus, the validity of early isotope work done on whole wood is in doubt.

An attempt has been made to investigate the presence of a possible thermodynamic thermometer between cellulose and lignin. Lignins are complex, crosslinked, three-dimensional polymers formed from phenolic units. The quantitative extraction of lignin from wood for isotopic determination appears very difficult if not impossible. However, taking into consideration that extractive-free wood is composed of cellulose, hemicellulose and lignin,

δD values of lignin may be calculated from δD values of extractive-free wood and cellulose.

If hemicellulose is assumed to have similar D/H ratios to cellulose and that lignin comprises 30% of extractive-free wood, the material balance of wood is expressed as extractive wood = 0.7 cellulose + 0.3 lignin. If the molecular formula of lignin is assumed to be the same as phenol (C_6H_7O), the total hydrogen in one gram of wood will be given by

$$H = 0.7\,\frac{\text{No. of hydrogen in cellulose}}{\text{molecular wt. of cellulose}} + 0.3\,\frac{\text{No. of hydrogen in lignin}}{\text{molecular wt. of lignin}}$$

$$= 0.0653. \qquad [48]$$

From the material balance of deuterium, δD values of lignin are calculated

$$0.0653\ \delta D_{NW} = 0.0432\delta D_{CN} + 0.0221\delta D_{lig} \qquad [49]$$

$$\delta D_{lig} = 3\delta D_{NW} - 2\delta D_{CN} \qquad [50]$$

where NW, CN and lig represent nitrated wood, cellulose nitrate and lignin respectively. The difference in δD value between cellulose and lignin is given by

$$\Delta\delta D = \delta D_{CN} - \delta D_{lig} = 3(\delta D_{CN} - \delta D_{NW}). \qquad [51]$$

Both δD values of lignin and $\Delta\delta D$ values are listed in Table 24. Table 25 shows various linear functional relationships among δD values of cellulose, lignin, and temperature. As is expected from the poor correlation between δD values of extractive-free wood and mean annual temperature, δD values of lignin, compared to cellulose nitrate, are poorly correlated with mean annual temperature. The fractionation between cellulose and lignin thus calculated shows a positive linear correlation with the mean annual temperature, which is contrary to thermodynamics. Thermodynamics predicts a decrease in fractionation as temperature increases. Furthermore, the correlation coefficient is lower than that of the cellulose nitrate-temperature relationship. Even the seemingly high correlation coefficient (0.8) of the ΔδD-temperature is mainly due to the effect of the high correlation between cellulose nitrate and temperature. A better knowledge of the chemical formula of lignin would not likely improve the correlation between ΔδD and temperature. Thus, the presence of a thermodynamic thermometer in cellulose-lignin pair is not guaranteed.

2.5 Isotopic Comparison between Nitration of Cellulose (WCCN) and Direct Nitration of Wood (WCN)

There are two ways of obtaining cellulose nitrate; one is the direct nitration of wood followed by acetone dissolution (WCN) and the other is the nitration of cellulose which has been extracted from wood by the sodium chlorite method (WCCN). The preliminary result of nitration of WS1 has shown that cellulose nitrates prepared by the direct nitration of wood are systematically more depleted in deuterium than those prepared by the nitration of cellulose extracted by the sodium chlorite method. δD values of cellulose nitrates prepared by the direct nitration of wood mills of WS1 are attached in Appendix 3. The precision of these data is ±3°/₀₀ whereas that of cellulose nitration is ±2°/₀₀. The linear regression estimate between the cellulose nitrated by WCN method and mean annual temperature is given by

$$\delta D_{WCN} = 7.5t - 163°/_{\circ\circ}. \qquad [52]$$

The correlation coefficient is 0.69 which is less than that of δD_{WCCN}-temperature relationship (0.8).

The low δD values of cellulose nitrate by the WCN method and the poor correlation with temperature may be attributed to the presence of impurities such as coloring matter and some type of hemicellulose. Cellulose nitrates prepared by direct nitration of wood are light brown in color indicating the presence of coloring matter and they are not as fluffy as nitrated cellulose. Since lipid fractions and lignins are drastically depleted in deuterium even very small amounts of these incorporated in the cellulose phase will reduce the overall δD values of cellulose nitrate.

Nevertheless, the similarity in temperature coefficients of cellulose nitrate-mean annual temperature relationships between two different nitration methods suggests that two methods measure essentially the same isotopic composition of non-exchangeable CH hydrogen in cellulose. However, reproducibility of nitration of cellulose appears better than that of direct nitration of wood. For this reason, all other cellulose nitrates were prepared by nitrating cellulose extracted by the sodium chlorite method.

2.6 Hydrogen Analysis of Trees from Various Localities

Table 26 contains cellulose δD values of trees from various growth sites in North America. Thin radial sections of tree ring series ranging from 15 to 60 years were used for the isotopic determinations. Average values of δD were obtained by taking long series of tree rings so that seasonal and yearly fluctuations are filtered out. Care was taken to exclude the juvenile rings in any samples to eliminate the possible problems associated with them. The functional relationship between δD values and mean annual temperature at the growth sites is found to be linear. The linear regression equation is found to be

$$\delta D = 6.0t - 130°/_{\circ\circ}. \qquad [53]$$

The correlation coefficient is 0.99 indicating there is a strong linear correlation between mean annual temperature and δD values in cellulose of trees. These data were combined with data selected from the literature (28,31) and plotted in Fig. 22. Selection was made on the basis

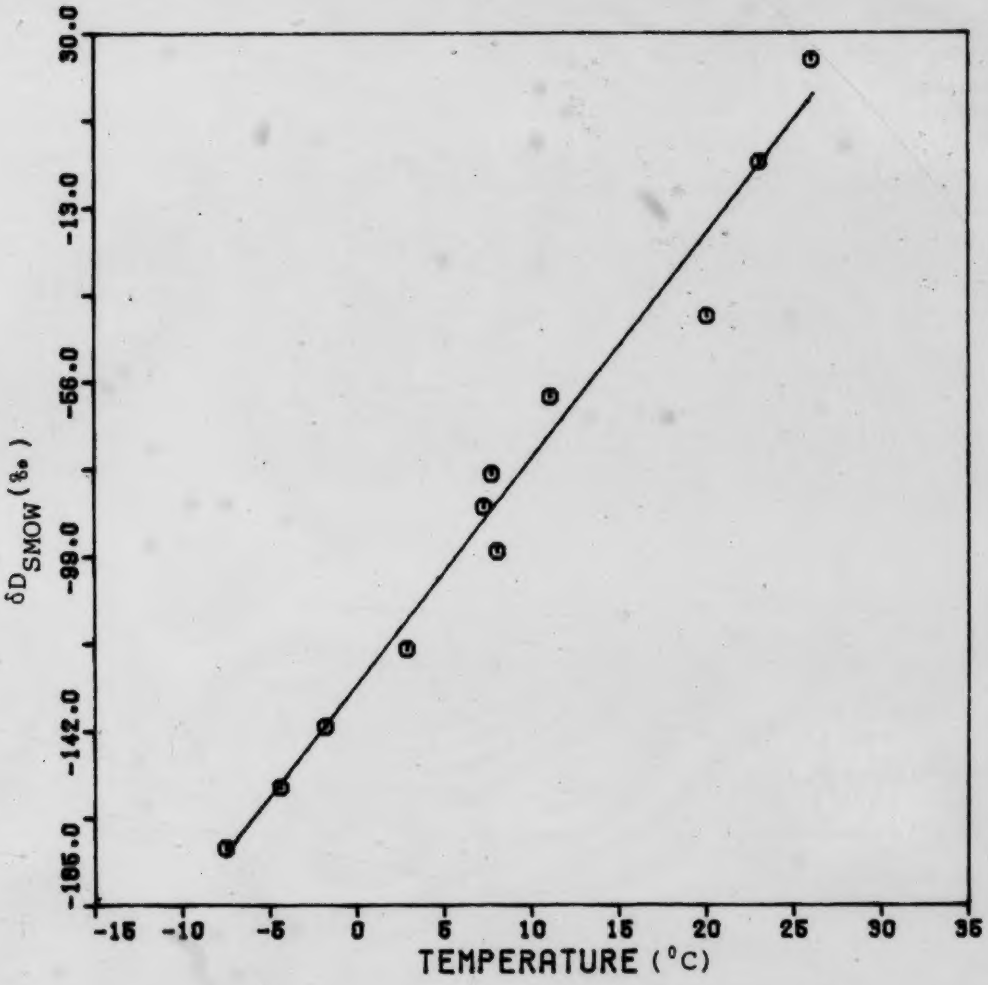

Figure 22. The relationship between δD values of tree rings from various sites in North America and mean annual temperatures. Each δD value represents the average over several years.

Table 26. δD Values of Cellulose Nitrate in Trees from Various Localities in North America and Mean Annual Temperatures at the Sites

Location	δD(°/∘∘ SMOW)	Temperature °C
1. Grant Grove, California	-60	11
2. Edmonton, Alberta	-122	2.8
3. Fort Vermillion, Alberta	-141	-1.9
4. Yukon I (Decoeli)	-156	-4.5
5. Yukon II	-171	-7.6
6. Vancouver Island, B.C.	-70	9.9
7. Oconto, Wisconsin	-87	7.2
8. Miami, Florida	-2	23
9. Spring Green, Wisconsin	-79	7.7
10. Houston, Texas	-40	20
11. Kalamalka, B.C.	-98	8
12. Puerto Rico	23	26

of climatic records being available so that mean annual temperatures could be calculated. Data from Epstein et al (31) were corrected by adding 5°/∘∘ to compensate for the difference in δD values obtained by the two nitration methods. The linear regression equation obtained from this reinforced hydrogen isotope data is given by

$$D = 5.5t - 130°/_{\circ\circ} . \qquad [54]$$

Equations [53] and [54] may be compared with equation [55] which describes the relationship between mean annual air temperature and mean annual δD values of meteoric water (15).

$$\delta D = 5.6t - 100^{\circ}/_{\circ\circ} \cdot \qquad [55]$$

This equation was derived from the equation between $\delta^{18}O$ values of meteoric water and mean annual surface temperature assuming that meteoric water satisfies

$$\delta D = 8\delta^{18}O + 10^{\circ}/_{\circ\circ} \cdot \qquad [56]$$

The $\delta^{18}O$ data used in this derivation were collected from coastal and island stations in tropical and middle latitudes. Thus, the validity of equation [56] may be restricted to those coastal stations. Nevertheless, the temperature coefficients (5.5 and 6) thus obtained are close to that of the meteoric water equation [56]. This is in keeping with the finding of Epstein et al (31) who reported a linear relationship in δD values between cellulose nitrate and environmental water. The linear regression equation has a slope of 0.97, an intercept of $-22^{\circ}/_{\circ\circ}$ and a correlation coefficient of 0.98.

The fact that the temperature coefficient is almost the same as that of the meteoric water equation indicates that the isotopic composition of the soil water is the primary factor which controls the D/H ratio of cellulose non-exchangeable hydrogen in plants and that soil water utilized by plants is essentially the same as meteoric water. The intercept of $-130^{\circ}/_{\circ\circ}$ in the equation, compared to $-100^{\circ}/_{\circ\circ}$ of the meteoric equation indicates that the cellulose carbon-bound hydrogens preferentially concentrate the lighter isotope with respect to the environmental water by an average 30°/₀₀ which is close to the 22°/₀₀ reported by Epstein et al (31).

3. Oxygen Isotope Results

3.1 Relationship between Temperature and $\delta^{18}O$ Values of Tree Rings

$^{18}O/^{16}O$ ratios of cellulose from samples WS1, WS2 and WS3 were measured. Each tree was divided into five-year groups of growth rings. Thus, the $\delta^{18}O$ value of each group represents the mean $\delta^{18}O$ value of the five consecutive tree rings. $\delta^{18}O$ values of cellulose from WS1, WS2 and WS3 are plotted in Fig. 23 together with mean annual temperature. In contrast to hydrogen, there seems to be no significant shift in $\delta^{18}O$ values between the juvenile and mature sections of growth rings for any of the three spruce samples. However, functional analysis of the oxygen isotope data with various climatic parameters were carried out only for mature rings to eliminate any such effects.

To investigate possible functional relationships between oxygen isotopic data and temperature, linear regression analysis was carried out between $\delta^{18}O$ values in cellulose of tree rings and various groups of temperature. The results are shown in Tables 27 and 28.

In general, $\delta^{18}O$ values of cellulose do not correlate as well as δD values with all temperature groups

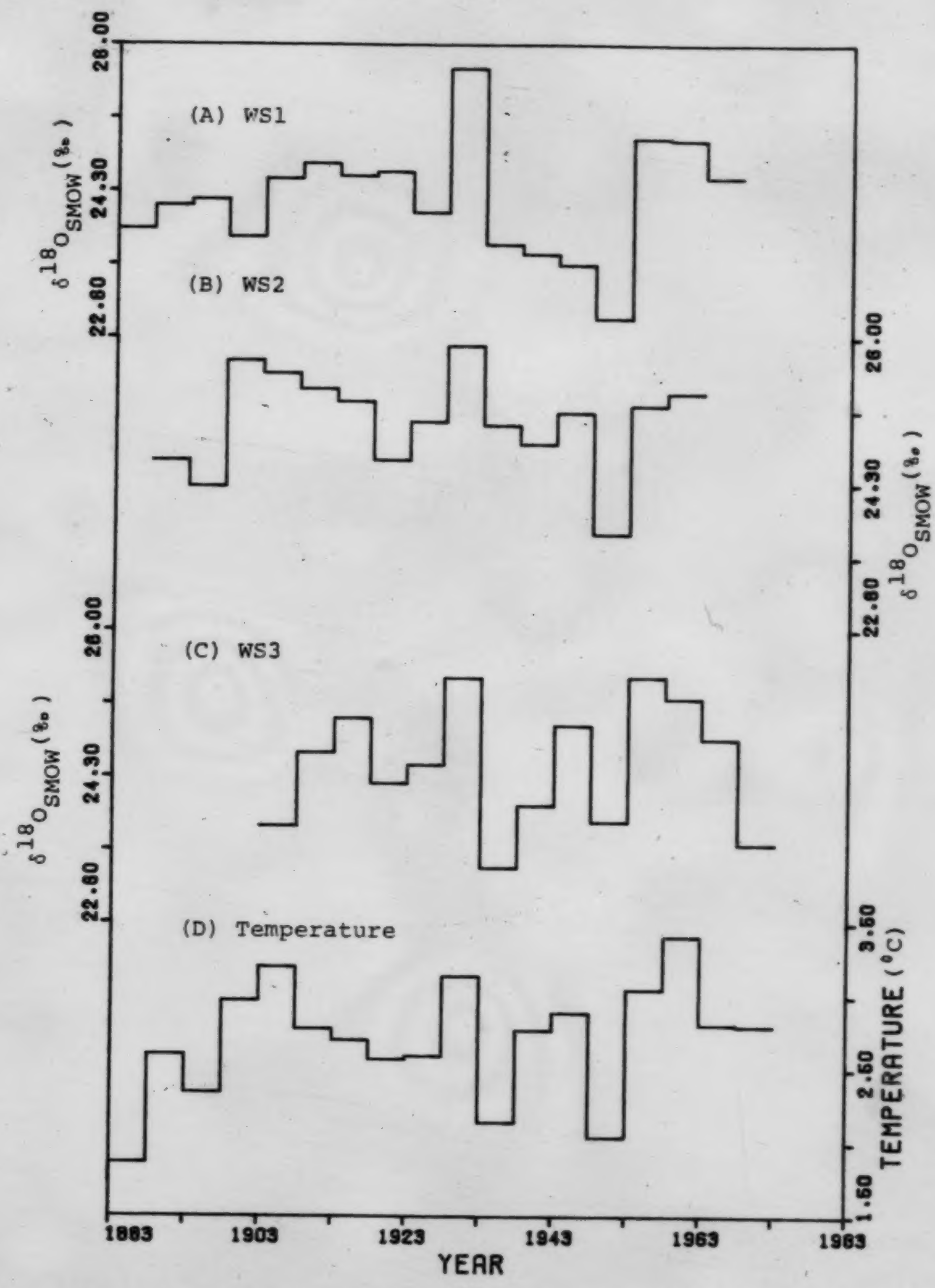

Figure 23. Comparison between $\delta^{18}O$ values of cellulose and mean annual temperature (September n-1 to August of contemporary year (n)). A) WS1, B) WS2, C) WS3, D) Mean annual temperature.

Table 27. Correlation between $\delta^{18}O$ Values of Cellulose and Various Annual Temperatures (t_a)

5-year Temperature Group	Relationship	CC*
Sample WS1		
Previous Year (Sept-Aug)	$\delta^{18}O = (1.36\pm0.41)t_a+(20.5\pm1.15)$	0.71
Contemporary Year (Sept-Aug)	$\delta^{18}O = (0.999\pm0.35)t_a+(21.5\pm0.97)$	0.66
Contemporary Year (Jan-Dec)	$\delta^{18}O = (1.22\pm0.46)t_a+(20.9\pm1.3)$	0.62
Sample WS2		
Previous Year (Sept-Aug)	$\delta^{18}O = (1.03\pm0.28)t_a+(22.2\pm0.77)$	0.76
Contemporary Year (Sept-Aug)	$\delta^{18}O = (0.69\pm0.27)t_a+(23.1\pm0.78)$	0.62
Contemporary Year (Jan-Dec)	$\delta^{18}O = (0.70\pm0.38)t_a+(23.1\pm1.06)$	0.51
Sample WS3		
Previous Year (Sept - Aug)	$\delta^{18}O = (1.47\pm0.39)t_a+(20.4\pm1.0)$	0.77
Contemporary Year (Sept-Aug)	$\delta^{18}O = (0.86\pm0.40)t_a+(22.2\pm1.1)$	0.56
Contemporary Year (Jan-Dec)	$\delta^{18}O = (0.79\pm0.58)t_a+(22.3\pm1.6)$	0.40

CC* = Correlation coefficient

Previous year (Sept-Aug) represents mean annual temperature measured from September of the n-2 year to August of the n-1 year.

Contemporary year (Sept-Aug) represents mean annual temperature measured from September of the n-1 year to August of the contemporary year (n).

Contemporary year (Jan-Dec) represents mean annual temperature measured from January to December of the contemporary year (n).

Table 28. Correlation between $\delta^{18}O$ Values of Cellulose and Various Seasonal Temperatures ($\delta^{18}O$ = at+b)

Sample	5-year Temperature Group	Slope °/₀₀/°C	Intercept °/₀₀	CC*
WS1	Winter (Dec-Mar)	0.33±0.16	27.7±1.7	0.52
	Summer (Apr-Aug)	1.27±0.45	8.5±5.6	0.64
WS2	Winter (Dec-Mar)	0.27±0.13	27.8±1.4	0.53
	Summer (Apr-Aug)	0.89±0.34	14.1±4.2	0.63
WS3	Winter (Dec-Mar)	0.31±0.18	27.7±1.9	0.48
	Summer (Apr-Aug)	0.32±0.5	20.3±6.2	0.21

CC* = Correlation coefficient

Winter (Dec-Mar) represents winter temperature measured from December of the n-1 year to March of the contemporary year (n).

Summer (Apr-Aug) represents summer temperature measured from April to August of the contemporary year (n).

examined. Nevertheless, $\delta^{18}O$ values of cellulose provide an insight into the role of various seasonal temperatures in determining the isotopic composition of tree rings. $\delta^{18}O$ values of WS1 have a slightly better correlation with growing season temperature than with winter temperature. This is contrary to the hydrogen isotope results where δD values are better correlated with winter temperature. Similarly, in the case of WS2, $\delta^{18}O$ values are slightly better correlated with growing season temperature than with winter temperature.

On the other hand, while the correlation between $\delta^{18}O$ values and winter temperature for WS3 is comparable with those of WS1 and WS2 the correlation with growing season temperature is much lower (0.21). The best correlation between $\delta^{18}O$ values and temperature was obtained when the 5-year mean annual temperature group was chosen to begin one year prior to the tree ring group. This was also found to be true for the hydrogen results. This suggests that the oxygen and hydrogen isotopic compositions of tree rings is determined mainly by the isotopic composition of stored soil water which has a large contribution from the previous year's precipitation at any given time.

However, the effect of seasonal temperatures on δD and $\delta^{18}O$ values of cellulose is somewhat different. Whereas δD values correlate better with winter temperature than with growing season temperature, $\delta^{18}O$ values have generally comparable correlations with winter and growing season temperature. This indicates a possible temperature-dependent fractionation of oxygen occurring in the growing season during photosynthesis. This possibility will be discussed in the next section.

3.2 Relationship between Precipitation and $\delta^{18}O$ Values of Tree Rings

Table 29 shows the correlation between $\delta^{18}O$ values of cellulose and the amount of precipitation. Even though all the correlation coefficients are low, it is apparent

Table 29. Correlation between $\delta^{18}O$ of Cellulose and Seasonal and Annual Precipitation

Sample	Season	Slope	Intercept	r
WS1	Annual (previous)	-0.44±0.19	30.8±2.9	0.56
	Annual (contemporary)	-0.34±0.2	29.3±3.1	0.44
	Winter (Dec-Mar)	-0.26±0.2	26.4±1.7	0.36
	Summer (Apr-Aug)	-0.08±0.075	26.2±1.8	0.32
WS2	Annual (previous)	-0.12±0.18	26.8±2.7	0.20
	Annual (contemporary)	-0.23±0.16	28.4±2.4	0.40
	Winter (Dec-Mar)	-0.27±0.14	27.3±1.1	0.54
	Summer (Apr-Aug)	-0.06±0.06	26.5±1.3	0.34
WS3	Annual (previous)	-0.45±0.18	31.2±2.7	0.62
	Annual (contemporary)	0.05±0.05	23.8±0.7	0.27
	Winter (Dec-Mar)	0.087±0.09	23.8±0.73	0.30
	Summer (Apr-Aug)	-0.2±0.07	29.1±1.8	0.63

Note:

Annual (previous) presents mean annual temperature calculated from September of the n-2 year to August of the previous year (n-1).

Annual (contemporary) represents mean annual temperature calculated from September of previous year (n-1) to August of contemporary year (n).

that $\delta^{18}O$ values of cellulose are best correlated with mean annual precipitation of the previous year. In contrast to temperature, however, the amount of winter precipitation shows higher correlation with $\delta^{18}O$ values than does the amount of growing season precipitation. This difference in the correlation of $\delta^{18}O$ with temperature and precipitation may suggest that in addition to $\delta^{18}O$ of stored soil water, summer temperature may play an important role in determining $\delta^{18}O$ value of cellulose. This was not found to be the case with D/H ratio values.

Based on the result that both δD and $\delta^{18}O$ of tree rings are a reflection of the isotopic composition of soil water, it is difficult to believe that the large difference in δD values between juvenile and mature sections of growth rings could be the result of climatic changes which occurred at the site since such changes would have affected both δD and $\delta^{18}O$ values of cellulose.

4. Relationship between Ring Width and Isotope Data

The use of ring width variations to construct past climate has received much attention in recent years (44,63). Furthermore, based on the fact that in many tree species, either early or late wood width is relatively constant and that the early and late wood generally have different isotopic composition (31,50), Wigley et al (97) have suggested that variations in isotopic composition in cellulose of tree rings may be, in part, a function of variations in ring width.

To find a possible relationship between ring width and isotopic compositions of cellulose, widths of single rings of the three spruce samples from Edmonton were measured. The tree ring widths can vary not only with fluctuations in environmental conditions, but also with systematic changes in tree age (44). Thus the normal standardization procedure in dendrochronology is to eliminate the ring width fluctuations associated with the changing age and geometry of the tree.

Standardization was carried out by transforming ring widths into ring-width indices. A growth curve was constructed by taking an 11 year moving average of the ring width data. The measured ring widths (Wt) were then converted to ring-width indices (It) by dividing each width for year (t) by the expected growth (Yt),

$$It = Wt/Yt. \qquad [57]$$

Division by the expected growth removes the trend in growth and scales the variance so that it is approximately the same throughout the entire length of the time series. Table 30 shows the equivalent rings. It is apparent that there is no significant correlation between the isotopic composition (either oxygen or hydrogen) and ring width for the three tree samples analyzed.

In the case of correlation between total ring width and δD values of the equivalent rings, total ring sequences including juvenile rings have a negative correlation whereas mature ring series have a positive but lower correlation. However, the high correlation coefficient of total ring sequences has no physical meaning. It is the direct result of the fact that the variation in δD of total ring sequence is largely due to the juvenile isotope effect whereas variations in δD of juvenile and mature rings are small.

Table 30. Correlation between Ring Width and Isotopic Composition in Cellulose

	W vs δD $\delta D = aW+b$		W vs $\delta^{18}O$ $\delta^{18}O = a'W+b'$	
Sample	Slope (°/oo/W)	r	Slope (°/oo/W)	r
WS1	(1) 23±23	0.25	1.7±1.3	0.32
	(2) 2.1±0.9	0.07	2.1±1.7	0.36
WS2	(1) 29±20	0.39	2.2±0.9	0.56
	(2) 8.7±15	0.19	-2.9±1.2	0.6
WS3	(1) -13±15	0.25	-0.28±0.9	0.09
	(2) -1.3±8	0.05	-0.04±0.1	0.09

(1) Total ring series including juvenile rings

(2) Mature rings

W = Ring width in arbitrary units

When the juvenile rings are eliminated from analysis, the correlation coefficients decrease drastically. This effect is not shown in the correlation with oxygen because there is no systematic difference in $\delta^{18}O$ values between the juvenile and mature rings.

This result is contradictory to the findings of Mook et al (77) who reported a negative correlation between δD and ring width in an oak tree from Drente, Netherlands. It is however difficult to compare their work with the present study because they did not provide the detailed analysis. For example, it is not clear whether or not their ring width data were standardized and their growth rings included juvenile rings. The poor correlation between ring width and δD or $\delta^{18}O$ values of equivalent rings was also observed by Gray and Thompson (50). They measured early wood width, late wood width and total ring width for the entire section of a tree. Further, oxygen isotopic analyses were carried out on early and late wood from a number of selected rings from the tree. In contrast to Wigley et al (104), the maximum contribution of ring effect to the variation is estimated to be less than 10% of the total variation (50). When 5-year groups of rings were used, this ring effect tended to minimize even further. Table 31 shows the correlation between ring width indices and mean annual temperature. Whereas isotopic compositions of tree rings are well correlated with temperature, there appears to be no significant correlation between tree ring width and mean annual temperature.

Table 31. Correlation between Ring Width and Mean Annual Temperature

(W=aT+b)

Sample	Slope (W°C)	Correlation Coefficient (r)
WS1	0.12±0.07	0.42
WS2	0.12±0.1	0.3
WS3	-0.04±0.17	0.07

W = Ring width in an arbitrary unit. T = Temperature

5. Relationship between δD and $\delta^{18}O$ Values of Cellulose

It is well known that the $\delta^{18}O$ and the δD in meteoric waters are related by the simple equation (12),

$$\delta D = 8\ \delta^{18}O + 10°/_{\circ\circ}.$$

In general, the slope of 8 indicates the simple Rayleigh condensation processes under equilibrium conditions. The validity of this equation was further confirmed by Dansgaard (16) who obtained essentially the same equation by analyzing waters from northern hemisphere continental stations. However, it is also well known that both the slope and the intercept of this equation vary in different regions (54,16). For example, meteoric waters at stations such as Nord, Alice Springs, Pretoria and Whitehorse, have a slope of approximately 5. The reduced slopes are attributed to non-equilibrium evaporation from falling raindrops. Thus the actual size of the slope may provide important information about the degree of kinetic effect at the site of precipitation.

The same principle can be applied to the leaf water system. The leaf water of terrestrial plants is enriched ^{18}O compared to the soil water and precipitation (37,20). The enrichment is caused by evapotranspiration of leaf water. It is a direct function of the relative humidity. In order to demonstrate such a relationship between δD and $\delta^{18}O$ of leaf water, Epstein et al (28) measured the δD and $\delta^{18}O$ of the water vacuum distilled from various plant leaves. The slope of δD vs $\delta^{18}O$ of leaf waters turned out to be 2.5. The low slope was attributed to the effect of non-equilibrium evaporation on the δD and $\delta^{18}O$ of water in the plant accompanying evapotranspiration through the leaves of the plant.

The question is whether or not the cellulose, the final product of photosynthesis utilizing leaf water, preserves the same relationship between δD and $\delta^{18}O$ observed for meteoric water. For example, Libby et al (68) in their isotopic analysis of an 1800 year ring sequence obtained a linear relationship between δD and $\delta^{18}O$ of whole wood. The slope of the equation turned out to be 8, which was used as evidence that both δD and $\delta^{18}O$ of whole wood reflect those of meteoric water.

To resolve this problem, an attempt has been made to find a functional relationship between δD and $\delta^{18}O$ of cellulose extracted from the three spruce samples (see Table 32). The slopes of the linear equations thus obtained for the three samples are 2.3±13, 7±2.3, and 5.3±2.1 respectively. Despite the fact that the three

Table 32. Correlation between δD and $\delta^{18}O$ of Tree Rings ($\delta D = a\ \delta^{18}O + b^{\circ}/_{\circ\circ}$)

Sample	Slope	Intercept $^{\circ}/_{\circ\circ}$	Correlation Coefficient
WS1	2.3±1.3	-190±31	0.48
WS2	7.0±2.3	-297±57	0.70
WS3	5.3±2.1	-252±51	0.62

samples are from the same climatic region, the slopes of these three trees are not the same even taking into account the large uncertainties in the slopes.

Taking into consideration that the standard deviations of the slopes are large, the slopes of WS2 and WS3 may be regarded as essentially the same having a value of approximately 6. This may be compared with the slope of 7.7 observed for meteoric water in the Edmonton region (54). The difference in slope between cellulose and meteoric water may indicate the degree of kinetic effect due to evapotranspiration occurring on the leaf structures.

The slope of 2.3 observed for WS1 is more difficult to explain. In general, a low slope of δD vs $\delta^{18}O$ relationship is associated with differential enrichment in heavy isotopes (D and ^{18}O) due to the kinetic isotope effects in evapotranspiration in conjunction with low relative humidity. Considering that WS1 and WS2 are from essentially the same site, it is unlikely that relative humidity at the growth site of WS1 was much lower than that of the site of WS2. The fact that the δD values of WS1 are systematically lower than those of WS2 and WS3 indicates that the inconsistency in the slope of δD-$\delta^{18}O$ relationship between individual trees may be elucidated in the context of the source of water taken by the individual trees and the source of hydrogen and oxygen in tree rings.

First, the composition of soil water (mixing ratio of summer-winter precipitation) taken by individual trees may be different. Thus soil water taken by WS1 is mainly winter precipitation and its isotopic composition is relatively constant. Since relative humidity in the Edmonton region is relatively low and the $\delta^{18}O$ value of water is more sensitive to the kinetic effect due to fast evaporation, the slope of δD-$\delta^{18}O$ equation for WS1 would be small. Thus variation in δD and $\delta^{18}O$ values of WS1 is thought to be caused mainly by relative humidity variation in this region. To explain this, a hypothetical growth condition may be considered. When a tree stands on a slope, the water taken by the tree will be composed of mainly winter precipitation because most of summer precipitation will run off. On the other hand, water taken by a tree on flat or hollow ground will be a mixture of the winter and summer precipitation. Then even if the relative humidity at both sites is the same, the water taken by the two trees will show different slopes provided that summer and winter precipitation preserve different slopes. The effect of humidity on the slope will also be different for the two trees. For example, it may be expected that the slope for the former tree would be more sensitive to relative humidity than that of the latter which is refurbished with fresh summer water. Thus the slope of δD-$\delta^{18}O$ relationship exhibited by individual trees may be different and it may not be a simple function of relative humidity. This hypothetical consideration may not be far from the actual case reported here. In support of this, it is interesting to note that the δD values of WS1 are better correlated with winter temperature than either with summer or mean annual temperatures whereas WS2 and WS3 show an equal contribution from summer and winter temperatures.

Secondly, the poor correlation between δD and $\delta^{18}O$ of cellulose may indicate that if hydrogen and oxygen originate from different sources, D/H ratios and $^{18}O/^{16}O$ ratios of cellulose may be independent of each other and thereby represent two different sources of climatic information. For example, unlike hydrogen, oxygen isotopes may experience temperature-dependent fractionation during photosynthesis. Then the δD-$\delta^{18}O$ relationship of meteoric water would no longer exist in the cellulose system. This problem will be discussed further in the following chapter. Finally, it is not completely ruled out that the inconsistent slopes between individuals of the same species are an artifact of the poorly defined relationship between δD and $\delta^{18}O$ of cellulose.

6. Model Study of the Isotope Composition of Cellulose

As a result of this experiment, it is obvious that isotopic compositions in cellulose of tree rings are determined not by a single climatic parameter but by several parameters such as temperature and humidity. It is worth developing a model which describes the association of isotopic variations with variations in climatic factors.

In isotope modelling, one of the most important procedures is to investigate the source of isotopes in the material in question. A clear understanding of the source of isotopes may resolve the problem as to what the isotopic variation in cellulose reflects, e.g. isotopic composition of water, temperature variation at the time of photosynthesis, and isotopic composition of air etc. As for the source of hydrogen in cellulose, it is quite obvious that hydrogen comes from water because plants do not absorb organic material from the ground and the amount of hydrogen gas in air is negligible. Therefore, it is to be expected that the hydrogen isotopic composition of water will play a primary role in determining the D/H ratios of cellulose in plants. However, the source of oxygen in cellulose is not an obvious matter. These exist at least two possible pathways whereby oxygen can be fixed into cellulose via photosynthesis.

6.1 Oxygen Isotope Model

Cellulose oxygen could be derived from atmospheric CO_2 with or without equilibration with water. Another possible pathway involves derivation of oxygen from water. Several models based on these assumptions have been proposed to account for the oxygen isotopic composition of cellulose in plants. For example, Epstein et al (28) postulated that one-third of cellulose oxygen in terrestrial plants comes from the environmental water and the other two-thirds from the atmospheric CO_2 without equilibration with water:

$$\delta^{18}O_{cell} = \frac{2}{3}\,\delta^{18}O_{CO_2} + \frac{1}{3}\,\delta^{18}O_{H_2O}\ . \qquad [58]$$

By combining this with the well known meteoric water, equation [56], Epstein et al (28) obtained a relationship between δD and δ^{18}O of cellulose. The slope of δ^{18}O vs. δD of cellulose was calculated to be 24 whereas the measured slope of the relationship is 14 (see Fig. 24). The difference in slope between the model and the actual data is almost in the order of magnitude. The model developed by Epstein et al (28) is not in full agreement with the actual data collected by the same authors. On the other hand, Ferhi et al (34) proposed a model of oxygen isotopes in cellulose based on laboratory experimental results. The model developed for the isotopic composition of the cellulose of terrestrial plants is composed of 38% of leaf water and 62% of atmospheric CO_2 which is not in isotopic equilibrium with either soil water or leaf water:

$$\delta^{18}O_{cell} = 0.38\,\delta^{18}O_{lw} + 0.62\,\delta^{18}O_{CO_2} \qquad [59]$$

where cell, lw and CO_2 represent cellulose, leaf water and CO_2 respectively. The result is contrary to the result obtained by DeNiro et al (19) who have reported complete exchange of the CO_2 with leaf water in the plants of their experiment. They undertook laboratory experiments in which they grew two sets of wheat plants under conditions

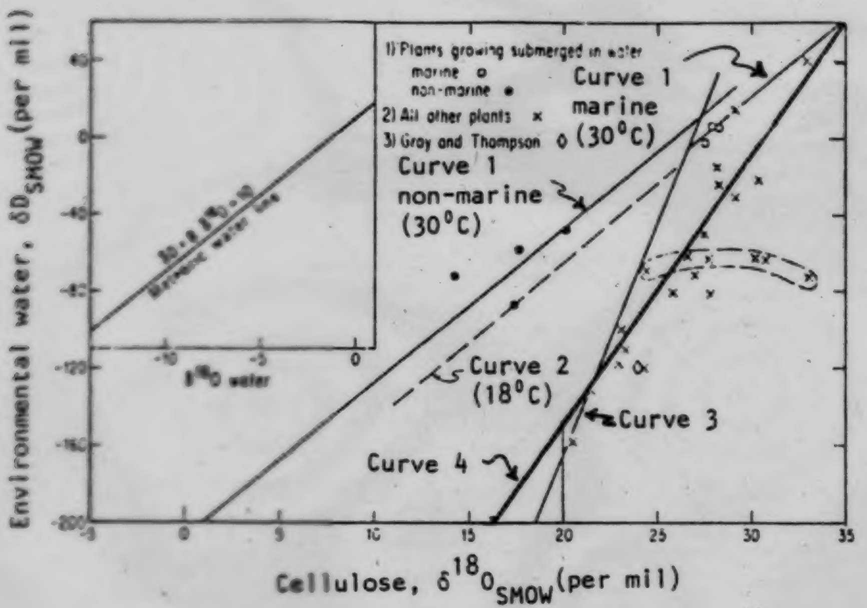

Figure 24. Relationships between the $\delta^{18}O$ of the cellulose (from both aquatic and terrestrial plants) and the δD of the meteoric water used by the plants (28). Note that the curve 4 is the simple linear regression curve for terrestrial plants whereas curve 3 was calculated by Epstein based on his oxygen isotope model:

Curve 3 $\delta D_w = 24\delta^{18}O_{cell} - 665$

Curve 4 $\delta D_w = 14\delta^{18}O_{cell} - 430$

similar in all respects except for a large difference in the $^{18}O/^{16}O$ ratios of the CO_2 supplied to them. Therefore, the difference in $\delta^{18}O$ values of the two celluloses can be attributed to the difference in $\delta^{18}O$ values of CO_2 gas supplied. This would indicate that the oxygen isotopic composition of atmospheric CO_2 influences that of cellulose in plants. In spite of the large difference in $^{18}O/^{16}O$ ratios of the CO_2 supplied to the two different wheat plants, the $^{18}O/^{16}O$ ratios of cellulose purified from the two sets differ by only a small amount. DeNiro and Epstein (19) thus concluded that the oxygen derived from CO_2 undergoes complete exchange with the oxygen of the water in the plant during photosynthesis and that the $^{18}O/^{16}O$ ratio of the leaf water is the primary influence on the $^{18}O/^{16}O$ ratio of the cellulose in terrestrial plants. The models proposed by Epstein et al (28) and Ferhi et al (34) fail to account for this phenomenon.

Here an attempt has been made to develop a model which accounts for the hydrogen and oxygen isotopic composition of cellulose in plants. Photosynthetic processes may be described schematically as

$$CO_2 + 2H_2O \rightarrow CH_2O + H_2O + O_2. \qquad [60]$$

Plant photosynthesis is an oxidation-reduction reaction between H_2O and CO_2, and uphill transfer of four hydrogen atoms from H_2O to CO_2 and can be divided into three parts (81):

1. Enzymatic transformation of H_2O to O_2

   $2H_2O \rightarrow 2H_2 + O_2$. [61]

2. Hydrogen transfer from an intermediate in enzymatic sequence 1. to an intermediate in enzymatic sequence 3 with the help of light activated chlorophyll.

3. Enzymatic transformation of CO_2 to CH_2O.

The first step, the enzymatic dehydrogenation of water was proven experimentally by Ruben et al (86) who demonstrated that the source of O_2 produced in photosynthesis is the water molecule and not CO_2. It is the third stage that involves incorporation of CO_2 into organic materials. Therefore, it is apparent that the primary source of oxygen in cellulose is CO_2. It is, however, unlikely that CO_2 could undergo direct reduction. Rather CO_2 is first bound during photosynthesis by a non-photochemical reaction to an organic acceptor. Ribulose Diphosphate (RDP) is found to be the primary acceptor of CO_2 and phosphoglyceric acid (PGA) is formed as the produce of this carboxylation. PGA thus produced reduces, through a photochemical process, in part to sugar and in part to the CO_2 acceptor, RDP. The oxygen atoms of CO_2 incorporated into PGA may undergo equilibration with H_2O by means of hydration as PGA reduces to carbohydrate through the Calvin cycle (81). The subsequent polymerization of glucose to form cellulose does not appear to provide any further opportunity for exchange of the oxygen derived from CO_2.

In this model, it is therefore assumed that the oxygen in cellulose originates from atmospheric CO_2 that is in complete isotopic equilibrium with water in the leaf and that the fractionation between the oxygen in CO_2 and that in the cellulose precursors in the Calvin cycle leading to cellulose formation is not temperature dependent. The equation for the oxygen isotope model is then defined as

$$\delta^{18}O_{cell} = A\delta^{18}O_{CO_2} + E. \qquad [62]$$

The equation has the form of a linear regression with slope of A and intercept of E. The slope A is the fractionation factor between the oxygen in cellulose and the oxygen in CO_2 undergoing isotopic equilibration with leaf water. Thus the actual value of A may depend upon the type of photosynthetic metabolism. This equation can be rewritten in terms of a fractionation factor $\alpha_{CO_2 - H_2O}$

$$\delta^{18}O_{cell} = A(1000 \ln\alpha + \delta^{18}O_{lw}) + E \qquad [63]$$

where α is fractionation factor between CO_2 and H_2O, and lw denotes leaf water. To find the actual value of A and E, the result of DeNiro et al (19) was used because this is the only experiment which measured $\delta^{18}O$ values of both leaf water and cellulose. The slope, A, turned out to be 0.62 and intercept 2.5‰. For this calculation, the fractionation factor $\alpha_{CO_2-H_2O}$ was calculated using the equation of Bottinga and Craig (5):

$$\alpha_t = [(5.112-0.214t+0.00041t^2) \times 10^{-3} + 1]\alpha_{25}. \qquad [64]$$

Thus the equation has the form

$$\delta^{18}O_{cell} = 0.62(1000 \ln\alpha_{CO_2-H_2O}+\delta^{18}O_{lw}) + 2.5°/_{oo}$$

$$= 620 \ln\alpha_{CO_2-H_2O}+0.62\delta^{18}O_{lw}+2.5°/_{oo}. \qquad [65]$$

Table 33 compares the $\delta^{18}O$ values calculated by different models with the experimental data obtained by DeNiro et al (19). As was mentioned earlier, all other models fail to account for $\delta^{18}O$ values of wheat plants in DeNiro's experiment.

Table 33. Comparison of Various Oxygen Isotope Models with Measured $\delta^{18}O$ Values of Cellulose

	$\delta^{18}O$ measured (19) °/oo	$\delta^{18}O_{SM}$ °/oo	$\delta^{18}O_{EM}$ °/oo	$\delta^{18}O_{FM1}$ °/oo	$\delta^{18}O_{FM2}$ °/oo
Experiment 1	26.8,27.9	26.7	21.9	20.3	25.1
Experiment 2	35.4,36.1	34.4	111.1	104.4	30.4

SM = present model

EM = Model of Epstein et al (28)

FM1 = Model of Fehri et al (34)

FM2 = Model of Fehri et al (34) revised

6.2 Isotopic Enrichment of the Leaf Water

In general, water in leaves of land plants are enriched in heavy isotopes (^{18}O and D) compared to water in branch or soil water (45,20,37). This enrichment is due to evapotranspiration. The variation in isotopic composition associated with evapotranspiration is given by a formula derived by Dongmann et al (20):

$$\frac{d\delta}{dt} = \frac{1}{\tau}\left(\delta - \{\varepsilon^* + \varepsilon_K + (\delta_A - \varepsilon_k)h\}\right) \qquad [66]$$

where $\varepsilon^* = \alpha - 1$, δ = the enrichment of leaf water relative to branch water, A = the enrichment of water vapor relative to branch water, ε_k = non-equilibrium separation factor and $\tau = \tau(n,j,h)$.

For steady state ($d\delta/dt=0$), the equation reduces to

$$\delta = \varepsilon^* + \varepsilon_k + (\delta_A - \varepsilon_k)h \qquad [67]$$

$$\delta_{lw} = \delta_{bw} + \varepsilon^* + \varepsilon_k + (\delta_A - \varepsilon_k)h \qquad [68]$$

where δ_{bw} is the isotopic composition of branch water. The uptake of soil water by the roots and its transportation to the branches causes no fractionation (37). Thus the isotopic composition of branch water in equation (68) can be replaced by those of soil water:

$$\delta_{lw} = \delta_{sw} + \varepsilon^* + \varepsilon_k + (\delta_A - \varepsilon_k)h. \qquad [69]$$

This equation describes the enrichment of leaf water in the heavy isotope due to evapotranspiration. DeNira et al (18), however, have shown that the $\delta^{18}O$ value of leaf water is influenced by that of CO_2. This effect could be a result of either oxygen isotope exchange between CO_2 and H_2O in the leaf or production of H_2O from photosynthetic processes in which CH_4O_2 dissociates into CH_2O and H_2O whose oxygen comes from CO_2. In natural systems, this CO_2 effect has not been noticed simply because $\delta^{18}O$ value of atmospheric CO_2 is relatively constant throughout the world (6) and the magnitude of the effect seems to be small ($0.6^{\circ}/_{\circ\circ}$). If the CO_2 effect is taken into consideration, the equation will have the form

$$\delta_{lw} = \delta_{sw} + \varepsilon^{*} + \varepsilon_{k} + (\delta_{A}-\varepsilon_{k})h + x\delta^{18}O_{CO_2}. \qquad [70]$$

The numerical value of x calculated from the result of DeNiro et al (19) turns out to be 0.0143. In this calculation, $\delta^{18}O$ value of inlet CO_2 was used for $\delta^{18}O_{CO_2}$ in the equation. Combining equations [65] and [70], the equation for the oxygen model becomes

$$\delta^{18}O_{cell} = 620 \ln\alpha_{CO_2-H_2O} + 0.62 \{\delta^{18}O_{sw} + \varepsilon^{*} + \varepsilon_{k} + (\delta_{A}-\varepsilon_{k})h + x\delta^{18}O_{CO_2}\} + 2.5. \qquad [71]$$

To test the validity of this equation, a number of parameters such as ε^{*}, ε_{k}, δ_{A} and h are required. The values of ε^{*} at different temperatures are well defined.

For example, at normal temperature ε^* is $9°/_{oo}$ (16). The kinetic separation factor ε_k is more difficult to estimate. The value of ε_k depends upon the type of aerodynamic boundary layer at which the kinetic separation occurs (20). The value of ε_k appears also to be species dependent. For example, deciduous trees such as oak and birch have ε_k value of about $15°/_{oo}$ whereas coniferous trees like fir and spruce have ε_k value of $24°/_{oo}$ (39). As for the value of A, it is important to note that there are two sources of water vapor in the environment of the leaf (20): well mixed water vapor from elsewhere and the water vapor from evapotranspiration of the surrounding plant and soil. Thus two limiting cases can be considered. The first is the case where the leaf is isolated from other vegetation. Furthermore, assuming that atmospheric water is in equilibrium with soil water, the A is determined by the equilibrium separation ε^*

$$\delta_A = -\varepsilon^* = -9°/_{oo} \text{ (at 25°C)}.$$

In the second case, the water vapor near the leaf originates exclusively from evapotranspiration. Under steady state conditions, the water vapor has the same isotopic composition as the soil water. Thus $\delta_A = 0$. In natural systems, however, δ_A will lie between these two extreme values. For example, the δ_A value measured in the Edmonton region is $-7°/_{oo}$ (22). Forstel (36) has demonstrated rather constant δ_A values of about $-8°/_{oo}$ regardless of the species of trees. Using these parameters, the oxygen model is calibrated with the actual data obtained from laboratory experiments by DeNiro et al (19). Table 34 compares the $\delta^{18}O_{cell}$ measured with $\delta^{18}O_{cell}$ calculated from the model.

Table 34. Comparison between the Actual and Model Values of $\delta^{18}O$ of cellulose

	$\delta^{18}O_{cell}$ measured	$\delta^{18}O_{cell}$ calculated
Experiment 1	26.8, 27.9	26.8
Experiment 2	35.4, 36.1	35.4

The equation thus obtained is given as

$$\delta^{18}O_{cell} = 620\ln\alpha_{CO_2-H_2O} + 0.62(\delta^{18}O_{sw} + 25-23H + 0.0143\,\delta^{18}O_{CO_2}) + 2.5 \qquad [72]$$

where $\varepsilon = 9^{\circ}/_{\circ\circ}$, $\varepsilon_k = 16^{\circ}/_{\circ\circ}$ and $\delta A = -7^{\circ}/_{\circ\circ}$.

Unfortunately, DeNiro et al (19) did not keep the relative humidity constant in their experiment. The relative humidity varied from 30% at the beginning to 100% at the end. Thus the relative humidity of 77% is taken as the average during growth experiment, which appears to be a reasonable choice. The result obtained from the model is in full agreement with experimental results of DeNiro et al (19).

6.3 Hydrogen Isotope Model

In the case of the hydrogen model, it is assumed that there is no temperature dependent fractionation during photosynthesis. This assumption is based on the fact that the relationship between δD values of cellulose nitrate and mean annual temperature is very similar to that between δD values of meteoric water and mean annual temperature:

$$\delta D_{cell} = 5.5\ \bar{t}_a\ -130^{\circ}/_{\circ\circ} \qquad [73]$$

$$\delta D_{mw} = 5.6\ \bar{t}_a\ -100^{\circ}/_{\circ\circ} \qquad [74]$$

In particular, the almost identical slopes indicate that the isotopic composition of the source water is the primary factor which controls the D/H ratio of cellulose. Thus the equation for the hydrogen model is given as

$$\delta D_{cell} = \delta D_{sw} + \varepsilon^{*} + \varepsilon_k + (\delta_A - \varepsilon_k)h + E \qquad [75]$$

where E is the biological factor. To calibrate this hydrogen model, the data of DeNiro et al (18) was used. The parameters for this calibration are as follows:

$\varepsilon = 80^{\circ}/_{\circ\circ}$ (16)

$\varepsilon_k = 14^{\circ}/_{\circ\circ}$ (73)

$E = 2^{\circ}/_{\circ\circ}$

$h = 77^{\circ}/_{\circ\circ}$.

δD_{cell} thus calculated are in excellent agreement with the measured values (see Table 35).

Table 35. Comparison between the Measured and Calculated D/H Ratios of Cellulose

	δD measured	δD calculated
Experiment 1	-13°/₀₀	-14°/₀₀
Experiment 2	-15°/₀₀	-14°/₀₀

The equation thus obtained is given by

$$\delta D_{cell} = \delta D_{sw} - 80h + 92. \qquad [76]$$

6.4 Calibration of the Isotope Models with Natural Systems

Finally, the validity of the models must be checked with the data from natural systems. For this purpose, a number of trees from various sites in North America were examined. Table 36 contains all the climatic information necessary for model calculations. Temperature is the mean daytime temperature during the growing season (usually April to August). Most of the climatic information was obtained from meteoric stations near the growth sites (1,80,87,100). However, in some cases climatic information was not available for the exact sample site. In this case, the data from the nearest meteorological station were used with corrections where applicable.

In applying this model to natural systems, some of

Table 36. List of Climatic and Isotopic Data for Model Calculations

Species	Location	T°C	RH	δ^{18}O°/oo water	δ^{18}O°/oo cell measured	δD°/oo water	δD°/oo cell measured
1. Red Mangrove	Puerto Rico	31	0.81	3.75	33	40	23
2. Red Mangrove	Miami	30	0.82	0.9	29.2	7	-2
3. Oak Tree	Houston	27	0.79	-4.3	30.5	-24	-40
4. Red Wood	Grant Grove	25	0.5	-10.6	30.2	-75	-60
5. Cedar	Vancouver	24	0.83	-7.6	26.0	-53	-70
6. Box Elder Tree	Madison, Wisconsin	24	0.73	-5.3	29.6	-32	-39
7. Hard Maple	Spring Green, Wis.	24	0.75	-9.9	26.6	-69	-79
8. White Birch	Oconto, Wis.	22	0.85	-9.4	25.8	-65	-87
9. Coutter Pine	Kalamalka, B.C.	20	0.63	-13.4	27.8	-97	-98
10. Coutter Pine	Mt. Whitney, Calif.	20	0.40	-14.5	30.8	-106	-81
11. White Spruce	Edmonton, Alberta	18	0.59	-18	25.0	-137	-123
12. Spruce	Lac Des Roches, B.C.	17	0.79	-15.4	23.3	-113	-129
13. White Spruce	Mt. Decoeli, Yukon	14	0.62	-21	22.6	-159	-156
14. White Spruce	Kluane Lake, Yukon	15	0.67	-23	20.6	-174	-176

T = day time temperature during growing season

RH = relative humidity

the values of the parameters involved in the model equation must be chosen. For example, the kinetic separation factor ε_k for the oxygen model was chosen as 24°/oo (38) because most of the trees analyzed were conifers. The value of $\delta^{18}O_A$ is assumed constant at -8°/oo. The term $0.0143\delta^{18}O_{CO_2}$ becomes 0.6°/oo because the oxygen isotopic composition of atmospheric CO_2 is constant at 41°/oo throughout the world. Thus the working equation for oxygen model reduces to

$$\delta^{18}O_{cell} = 620 \ln\alpha_{CO_2-H_2O} + 0.62(\delta^{18}O_{sw}+33.6-32h) + E. \quad [77]$$

In the case of hydrogen, the parameters used in this calculation are as follows:

$\varepsilon^* = 80°/oo$
$\varepsilon_k = 24°/oo$
$\delta D = -80°/oo$
$E = -35°/oo$.

Hence the equation for the hydrogen model becomes

$$\delta D_{cell} = \delta D_{sw} + 104 - 104h - 35°/oo. \quad [78]$$

The equation for both oxygen and hydrogen are in excellent agreement with the actual data (Table 37). Fig. 25 shows the strong linear correlation between $\delta^{18}O_{cell}$ measured and $\delta^{18}O_{cell}$ calculated from the model. The correlation coefficient is 0.98. Fig. 26 shows the linear relationship

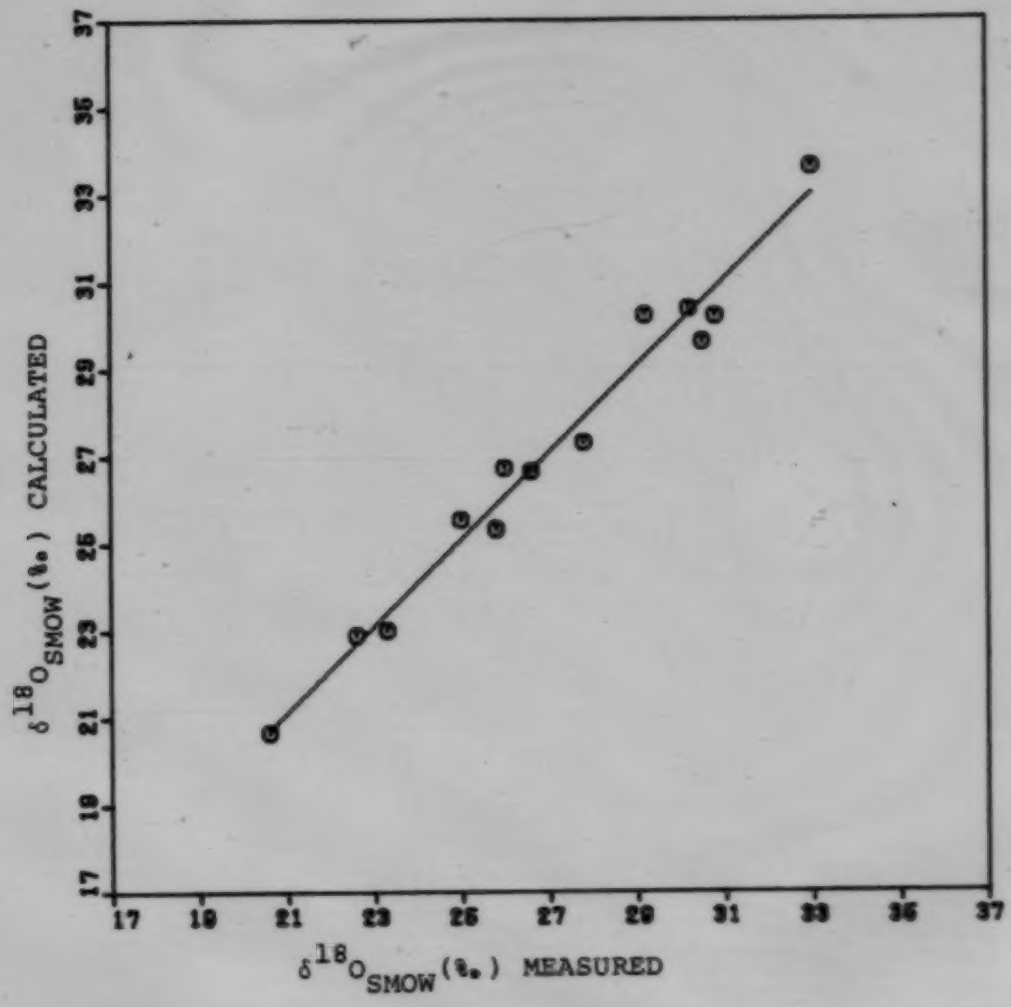

Figure 25. The relationship between $\delta^{18}O$ values of cellulose measured and calculated with the oxygen isotope model for trees from various growth sites in North America.

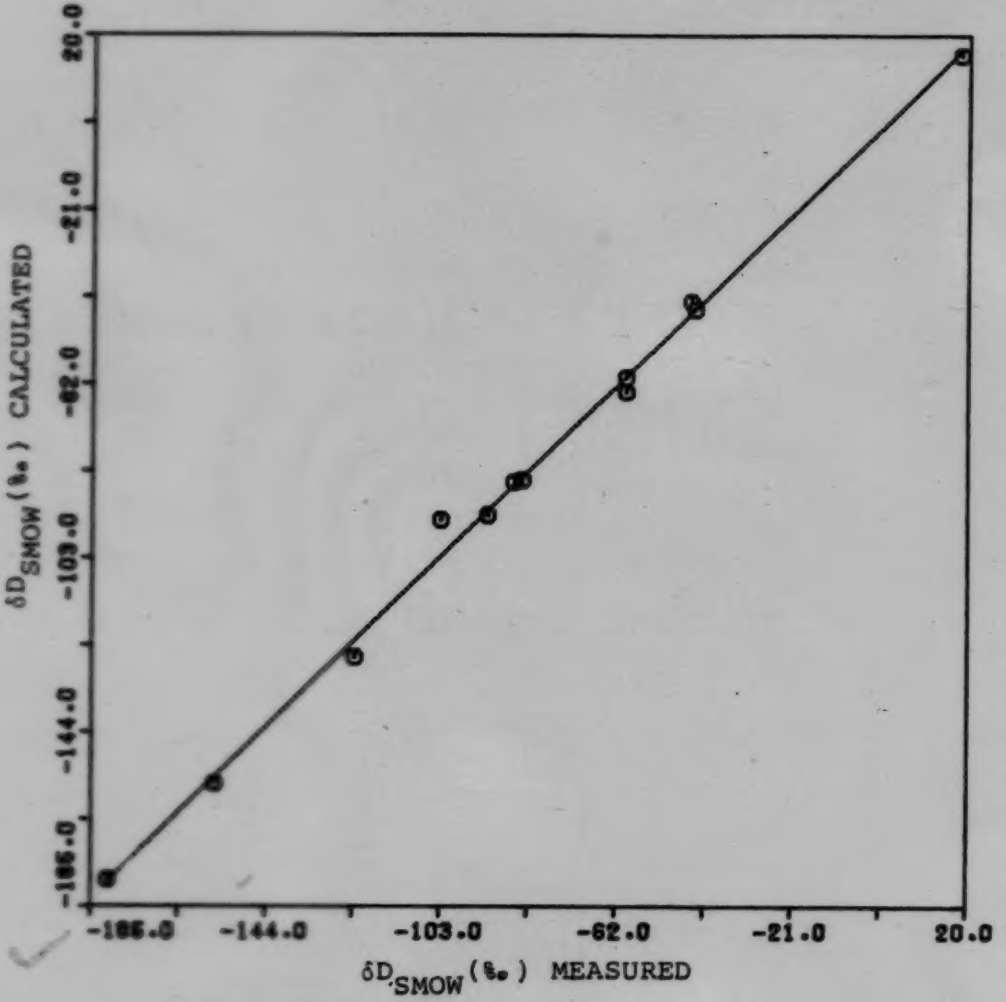

Figure 26. The relationship between δD values in cellulose nitrate measured and calculated using the hydrogen model developed for trees from various growth sites in North America.

Table 37. Correlation between various pairs of isotopic and climatic parameters

parameter pair	slope	intercept	correlation coefficient
δD measured vs δD model	1±0.02	-0.3±1.6	0.998
δD cellulose vs δD water	0.88±0.06	-13±6	0.974
δD cellulose vs summer temperature	10±1	-306±19	0.960
δD cellulose vs relative humidity	134±111	-174±79	0.329
δD cellulose vs $\delta^{18}O$ water	7±0.5	-3.6±6.02	0.974
δD cellulose vs $\delta^{18}O$ cell	14±2	-459±55	0.894
δD water vs summer temperature	11.2±0.8	-326±19	0.968
δD water vs relative humidity	238±110	-241±78	0.528
$\delta^{18}O$ model vs $\delta^{18}O$ model	0.93±0.05	1.6±1.5	0.981
$\delta^{18}O$ cellulose vs $\delta^{18}O$ water	0.38±0.08	31±1.1	0.788
$\delta^{18}O$ cellulose vs summer temperature	0.54±0.12	15.3±2.7	0.792
$\delta^{18}O$ cellulose vs relative humidity	-1.7±0.8	28.4±5.3	-0.066
$\delta^{18}O$ water vs summer temperature	1.39±0.1	-41.6±2.3	0.967
$\delta^{18}O$ water vs relative humidity	29.3±14	-31.1±9.7	0.527

between δD cell measured and δD cell calculated by the model. The correlation coefficient is 0.998. The large correlation coefficients for both oxygen and hydrogen models indicate that the assumptions made in this modelling are generally true.

To further illustrate the role of individual parameters in the model equations, a number of additional relationships are given in Table 37. δD values of cellulose have a strong linear relationship with that of soil water whereas $\delta^{18}O$ values of cellulose have poor correlation with those of soil water (see Figs. 27,28). In fact, δD values of cellulose even have an excellent correlation with $\delta^{18}O$ values of water (see Fig. 29). These indicate that δD values of cellulose mainly reflect δD values of soil water but $\delta^{18}O$ values of cellulose are influenced by other factors in addition to $\delta^{18}O$ values of soil water. In support of this, δD values of cellulose show good correlation with the growing season temperature (see Fig. 30), which can be attributed to the good correlation between δD_w and temperature (see Fig. 31). Despite the fact that $\delta^{18}O$ values of soil water are relatively well correlated with summer temperature (see Fig. 32), the $\delta^{18}O$ values of cellulose are less sensitive to the growing season temperature (see Fig. 33). There are a number of reasons for this poor correlation. First, the contribution of the $\delta^{18}O$ value of water to the $\delta^{18}O$ value of cellulose is only 62% whereas that of hydrogen is 100%. Second, the CO_2-H_2O

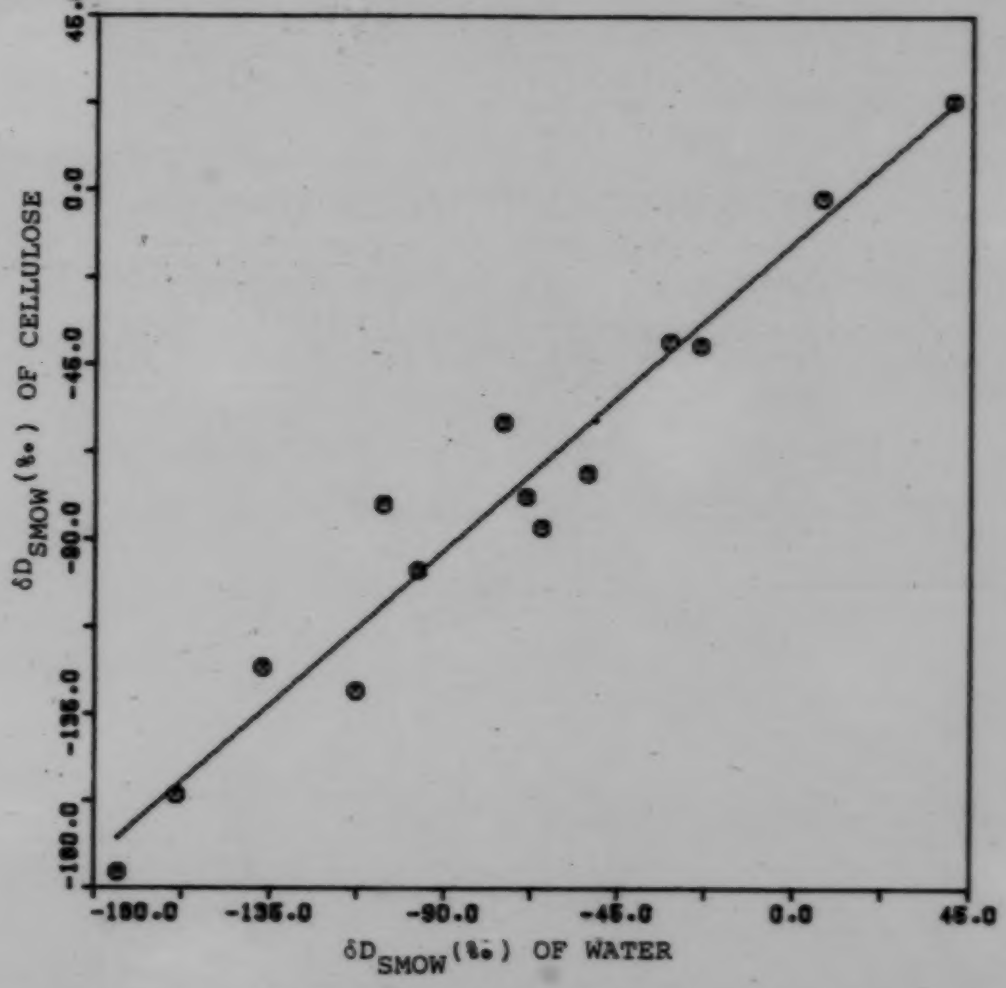

Figure 27. The relationship between δD values of cellulose nitrate in trees from various sites in North America and δD values of meteoric water used by the plants.

$\delta D_{cell} = (0.9 \pm 0.1)\delta D_{w} - (12 \pm 6^{\circ}/_{\circ\circ})$

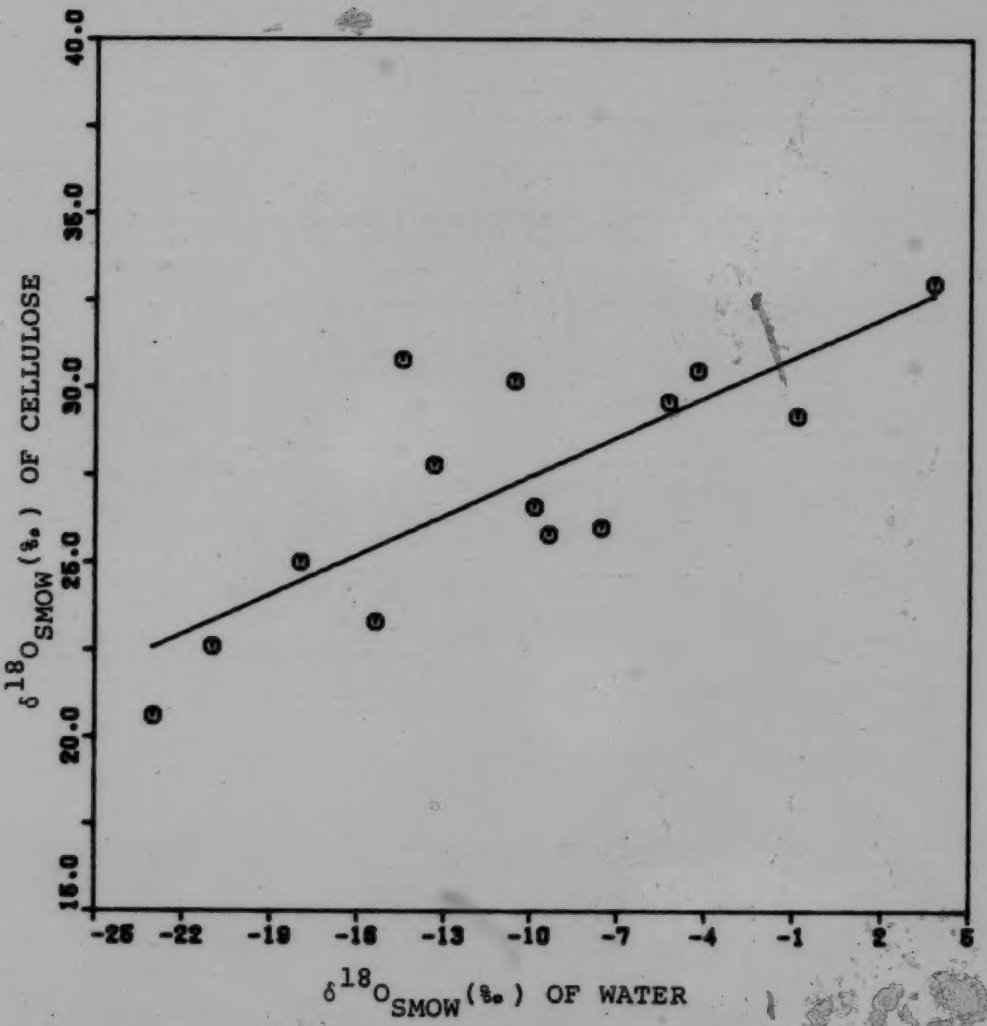

Figure 28. The relationship between $\delta^{18}O$ values of cellulose in trees from various sites in North America and $\delta^{18}O$ values of meteoric water used by the plants

$\delta^{18}O_{cell} = (0.38\pm0.08)\delta^{18}O_w + (31\pm1°/_{\circ\circ})$

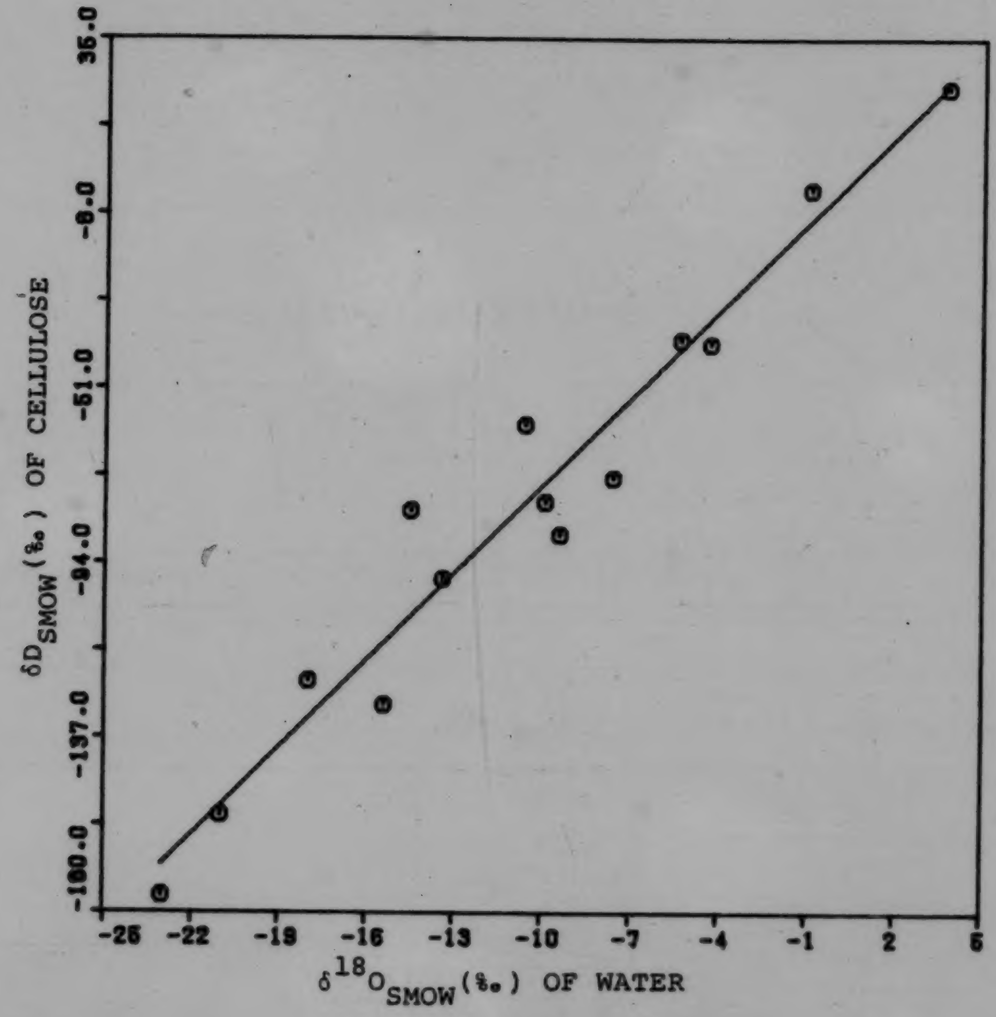

Figure 29. The relationship between δD values of cellulose nitrate in trees from various sites in North America and $\delta^{18}O$ values of meteoric water used by the plants.

$$\delta D_{cell} = (7.0\pm0.5)\delta^{18}O_w - (3.6\pm6.2)$$

Note that the slope is close to that of the meteoric water line.

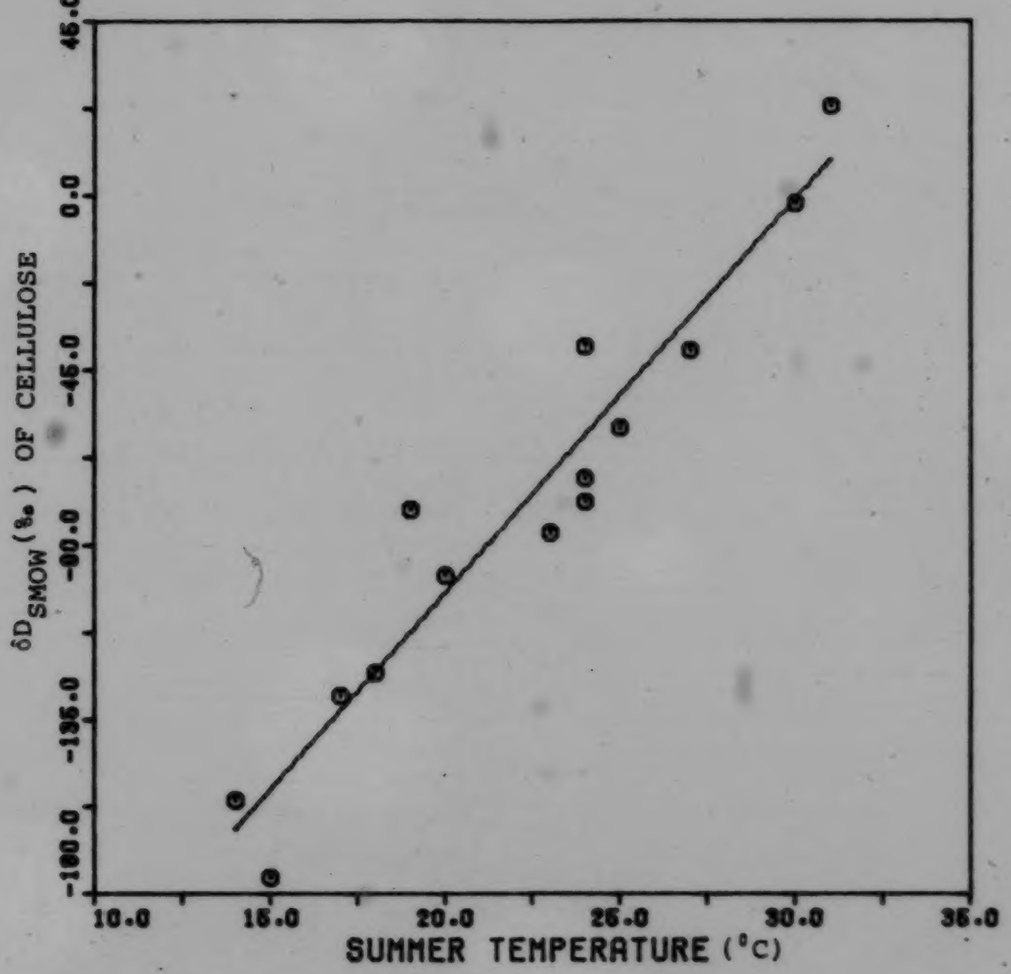

Figure 30. The relationship between growing season temperature and δD values of cellulose nitrate in trees from various localities in North America.

$$\delta D_{cell} = (10 \pm 0.9)ts - (306 \pm 19^{\circ}/_{\circ\circ})$$

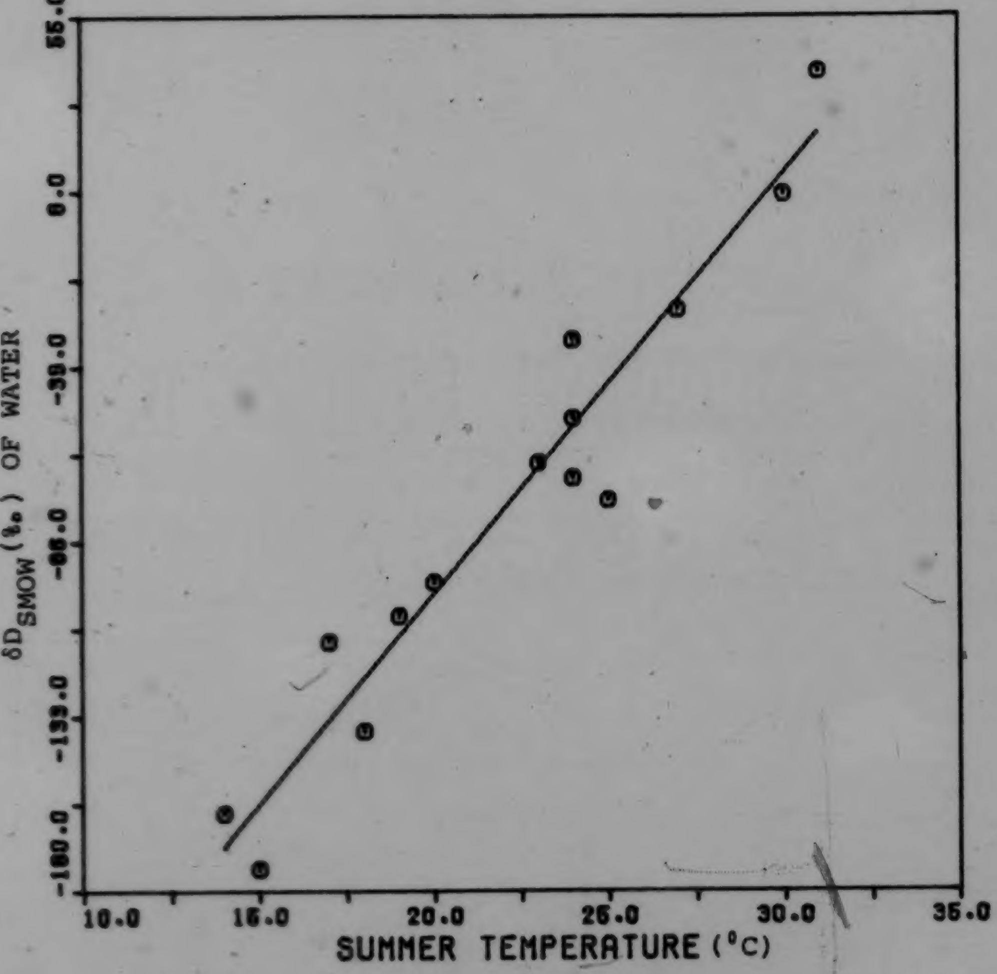

Figure 31. The relationship between growing season temperature and δD values of meteoric water.

$\delta D_w = (11.2 \pm 0.8)ts - (326 \pm 19°/_{\circ\circ})$

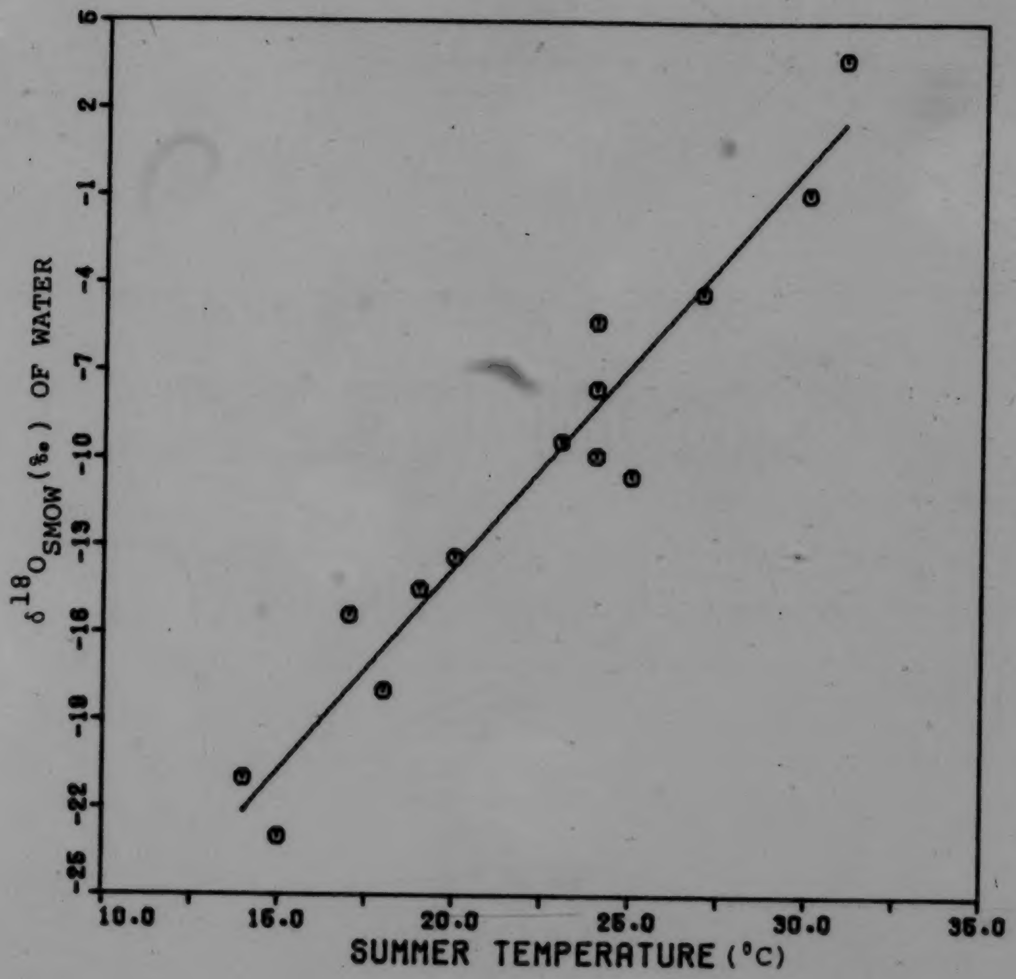

Figure 32. The relationship between growing season temperature and $\delta^{18}O$ values of meteoric water.

$\delta^{18}O_W = (1.4 \pm 0.1)ts - (42 \pm 2°/_{\circ\circ})$

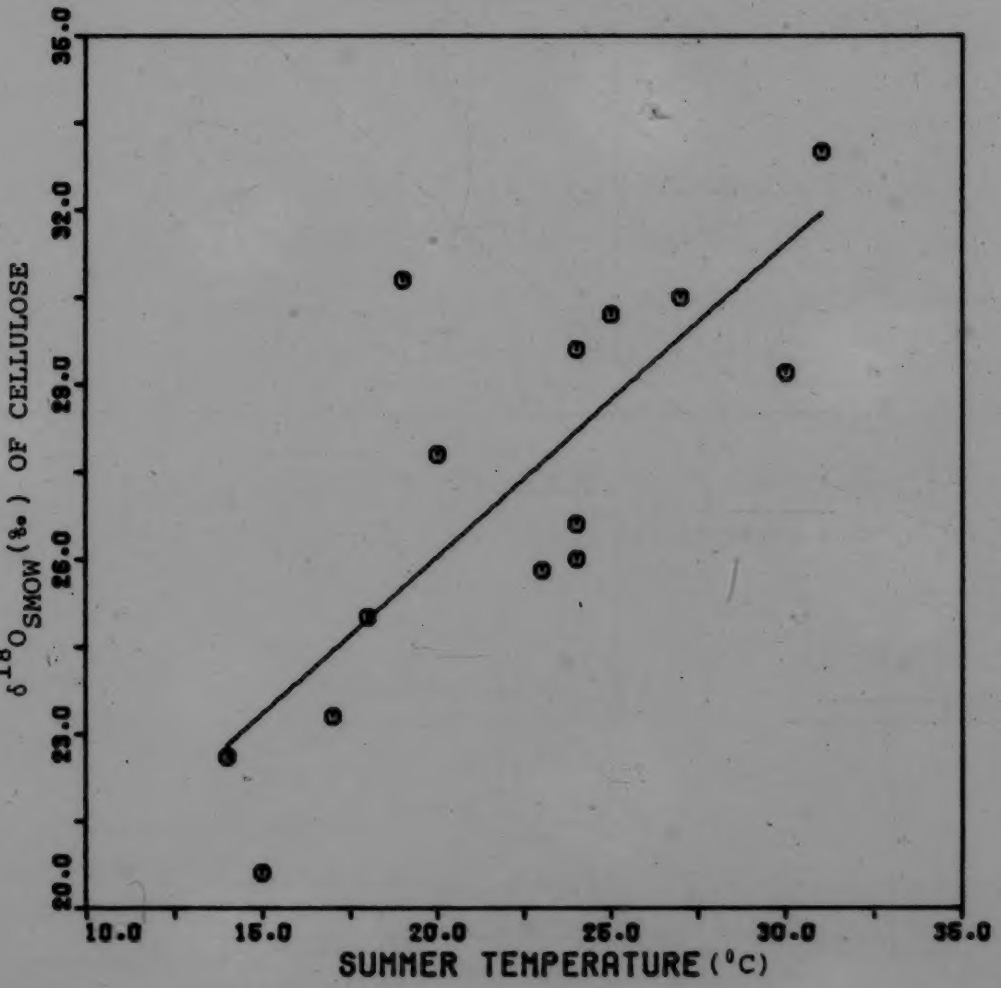

Figure 33. The relationship between growing season temperature and $\delta^{18}O$ values of cellulose in tree rings from various localities in North America.

$$\delta^{18}O_{cell} = (-.54\pm0.12)ts + (15.3\pm2.7°/_{\circ\circ})$$

fractionation factor has a negative correlation with the temperature whereas the regression coefficient between $\delta^{18}O$ value of water and temperature is positive. Finally, oxygen is sensitive to the kinetic effects of evapotranspiration. Thus $\delta^{28}O$ values of cellulose become less sensitive to the growing season temperature due to the conflicting effects of the growing season temperature on $\alpha_{CO_2-H_2O}$, $\delta^{18}O$ value of water and kinetic effect of evapotranspiration.

Fig. 34 shows the linear relationship between relative humidity and $\Delta\delta D$ which is the enrichment of cellulose relative to soil water defined by

$$\Delta\delta D = \delta D_{cell} - \delta D_{sw}. \tag{78}$$

The striking feature of Fig. 34 is the linearity between $\Delta\delta D$ (enrichment of cellulose with respect to soil water) and relative humidity. In sharp contrast to Epstein's finding that the deuterium enrichment of cellulose relative to environmental water is constant ($-22^\circ/_{\circ\circ}$), it is apparent that the enrichment of cellulose is a linear function of relative humidity. Thus if the difference in δD values between soil water and cellulose in tree rings is known, the relative humidity of the growth site can be obtained and vice versa.

However, it is interesting to note that both the δD and the $\delta^{18}O$ of cellulose in tree rings are poorly correlated with relative humidity (see Table 37). This may be due to the fact that unlike meteoric water, relative

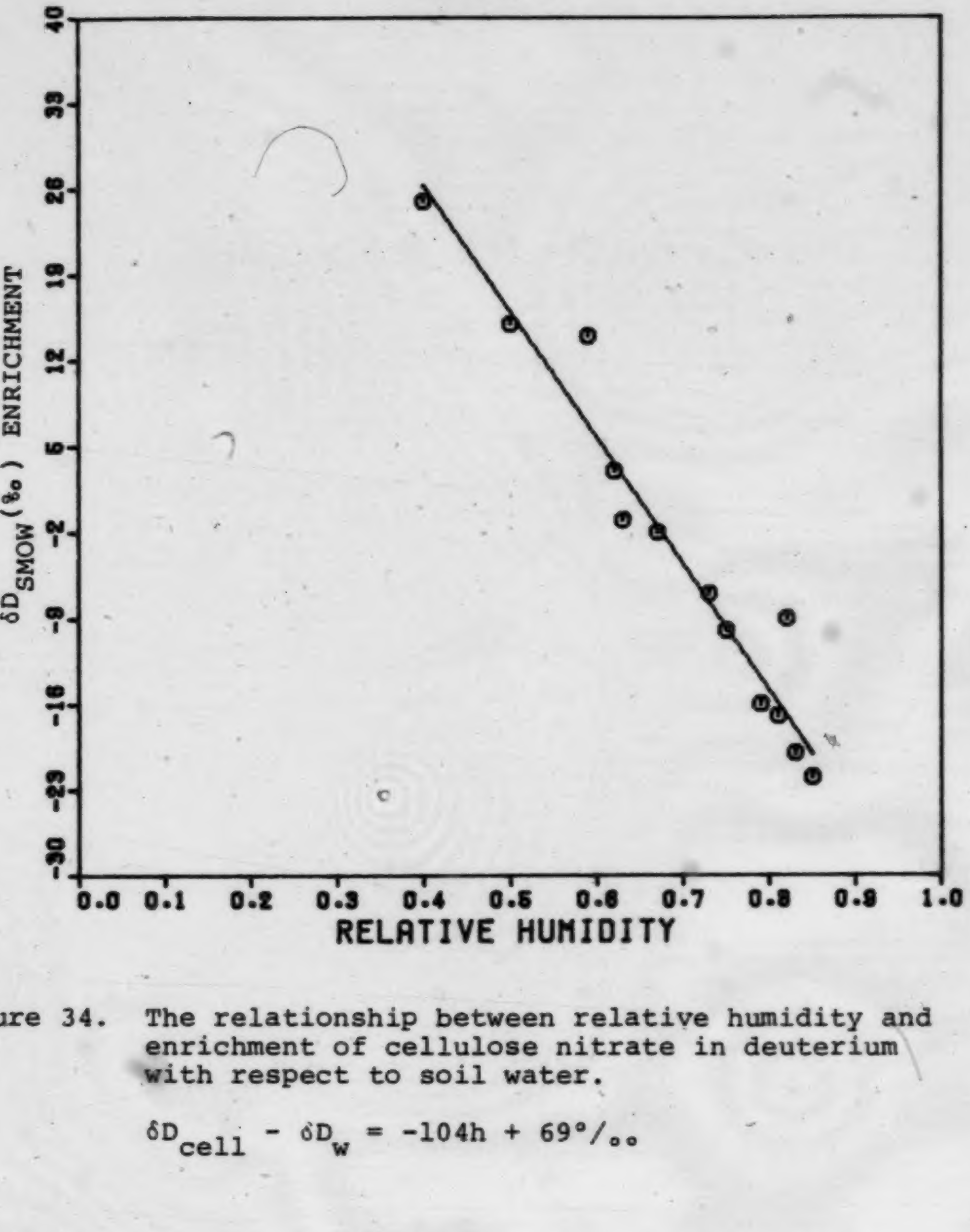

Figure 34. The relationship between relative humidity and enrichment of cellulose nitrate in deuterium with respect to soil water.

$$\delta D_{cell} - \delta D_{w} = -104h + 69^{\circ}/_{\circ\circ}$$

humidity has no significant functional relationship with geographic factors such as latitude and altitude, which is partly shown in poor correlations of relative humidity with either the isotopic composition of soil water or with air temperature. For example, air temperature, in general, decreases as the latitude or altitude increases in the northern hemisphere. However, no such generalization is found with relative humidity.

Despite the fact that oxygen isotope fractionation is sensitive to relative humidity, Gray et al (52) obtained linear relationship between $\delta^{18}O$ values of cellulose and mean annual temperature for various terrestrial and peat deposits. The virtually identical slopes shown by two different types of plants representing different humidity environments were attributed to the constant relative humidity effect by Gray and Thompson (52). However, it also may be interpreted as a lack of a functional relationship between relative humidity and $\delta^{18}O$ values of cellulose due to random distribution of relative humidity throughout North America. Thus the scatter of isotope data in Fig. 12 may be attributed mainly to random distribution of relative humidity throughout the regions.

However, when the isotopic variation of cellulose from a single tree is considered, the relative humidity plays an important role because variation in relative humidity at a given site appears to have a functional relationship with the change of air temperature at the site.

6.5 Relationship between δD Values and $\delta^{18}O$ Values of Cellulose inferred from the Model Study

From the models developed, it is obvious that the relationship between δD and $\delta^{18}O$ of meteoric water does not hold for cellulose in plants. Thus, a new relationship between δD and $\delta^{18}O$ of cellulose is sought. The equations used in this calculation are as follows:

$$\delta^{18}O_{cell} = 620 \ln\alpha_{CO_2-H_2O} + 0.62(\delta^{18}O_{sw} + 33.6-32h) + 2.5 \qquad [80]$$

$$\delta D_{cell} = 8\delta^{18}O_{sw} - 104h + 69 \qquad [81]$$

$$\delta D_{sw} = 8\delta^{18}O_{sw} + 10. \qquad [82]$$

First, the case where trees from various localities are involved is considered.

Eliminating δD_w and $\delta^{18}O_w$ yields

$$\delta D_{cell} = 13\delta^{18}O_{cell} + 152h-8000 \ln\alpha_{CO_2-H_2O} - 216. \qquad [83]$$

Since δD_{cell} has almost no functional relationship with relative humidity or the fractionation factor $\alpha_{CO_2-H_2O}$, the humidity term and fractionation factor can be regarded as constant with large random errors. If relative humidity and daytime temperature are arbitrarily chosen to be 70% and 25°C respectively, the equation becomes

$$\delta D_{cell} = 13\delta^{18}O_{cell} - 432. \qquad [84]$$

The linear regression equation describing δD and $\delta^{18}O$ of cellulose in trees from various sites in North America was calculated (see Fig. 35):

$$\delta D_{cell} = 14\delta^{18}O_{cell} - 459. \qquad [85]$$

The slopes of the two equations are identical within the error limits. Furthermore, the close agreement of the intercepts indicates that the models developed in this study do indeed account for the isotopic composition of cellulose in plants.

Even if there is no systematic geographical variation in relative humidity, the variation of relative humidity for a given site appears to have a functional relationship with the air temperature. Thus the relationship between δD and $\delta^{18}O$ values of cellulose in a series of tree rings from a single tree is likely different from the one obtained above. Figures 36 and 37 show the diurnal and daily variations of air temperature and relative humidity at Julich (37,20). From these figures, it is apparent that both the diurnal and daily variations of relative humidity are not constant and must be eliminated from the equation. Elimination of relative humidity reduces equation [81] to

$$\delta D_{cell} = 5.2\,\delta^{18}O_{cell} + 4.75\delta^{18}O_{sw} - 242. \qquad [86]$$

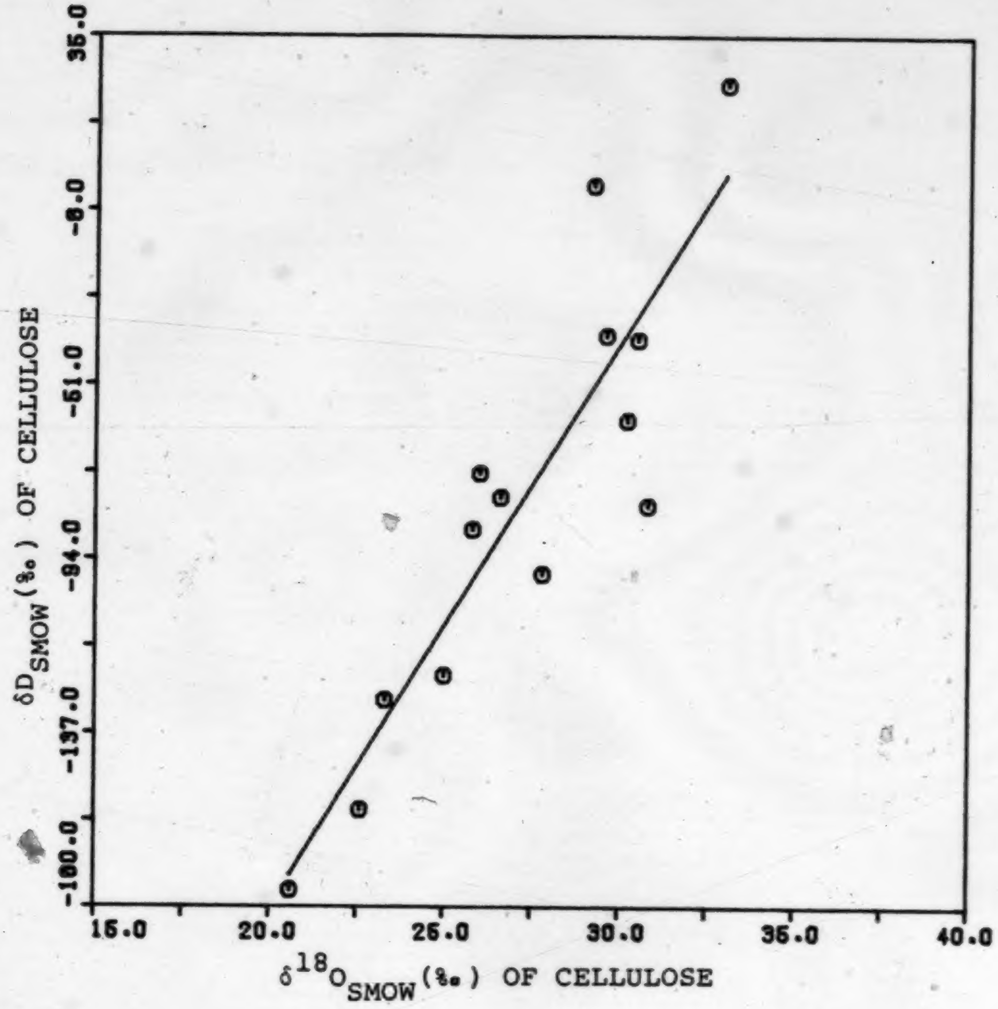

Figure 35. The relationship between the $\delta^{18}O$ values of cellulose and the δD values of cellulose nitrate in trees from various localities in North America.

$$\delta D_{cell} = (14\pm2)\delta^{18}O_{cell} - (459\pm55)$$

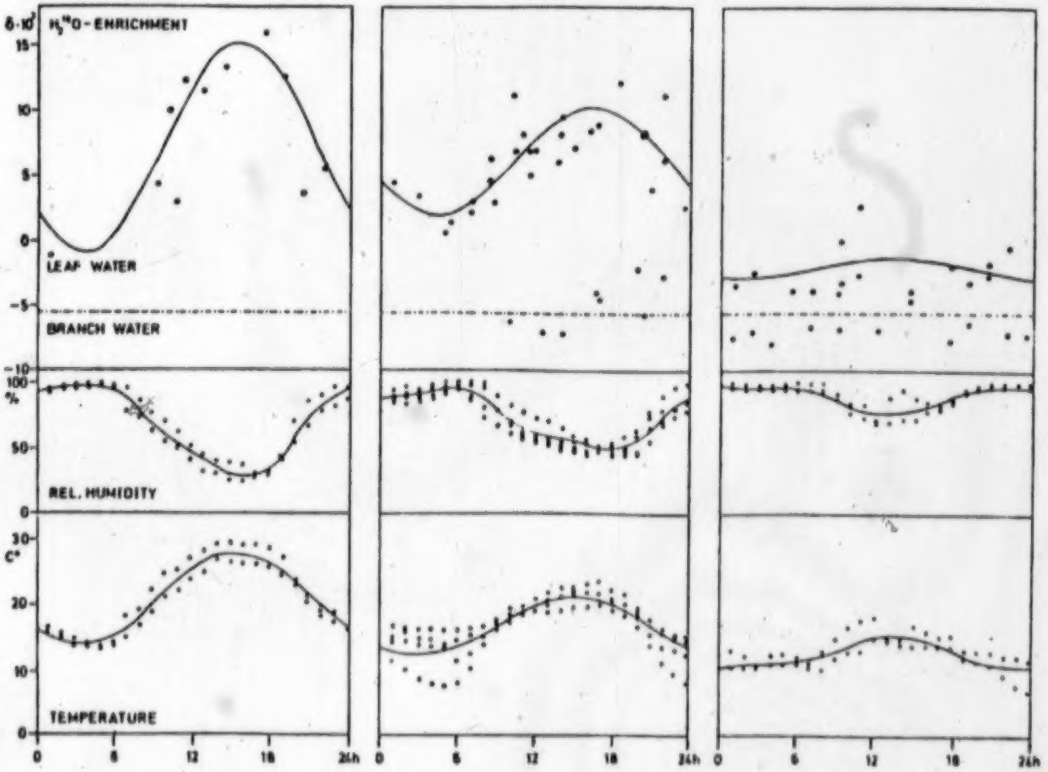

Figure 36. Diurnal rhythm of $H_2{}^{18}O$ enrichment in the leaves of a beech, relative to SMOW (20).

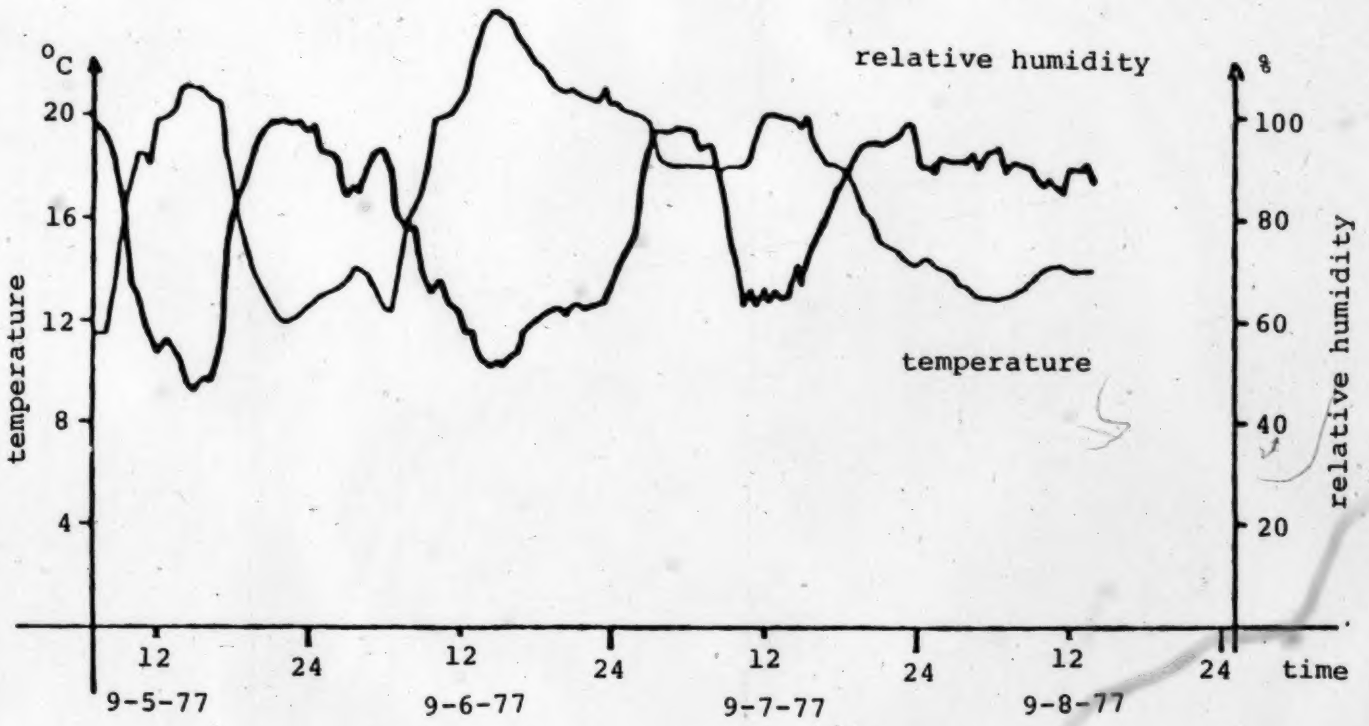

Figure 37. Temperature (thin line) and relative humidity (thick line) measured under standard conditions at the experimental site during the observation period (37).

Since the variation of $\delta^{18}O$ of soil water for a given site is relatively small and approximately one-half of $\delta^{18}O$ variation of cellulose, the equation reduces to

$$\delta D_{cell} = (5.2 \pm 2)\delta^{18}O_{cell} + E. \qquad [87]$$

In support of this result, the spruce trees from Edmonton have slopes of 2.3, 7 and 5.3 which lie within the range of the calculated slope.

6.6 The Best Climatic Information from Tree Rings

It is apparent that relative humidity influences both the hydrogen and the oxygen isotopic composition of cellulose in tree rings. However, the derivaton of humidity information from isotopic data of cellulose in plants appears to be unreliable and impractical. Despite the fact that the fractionation $\Delta\delta D$ between δD_{cell} and δD_w is well correlated with relative humidity, δD of cellulose has a poor correlation with relative humidity. This indicates that unless both δD of cellulose and δD of soil water are known, δD of cellulose cannot provide any reliable information about relative humidity. In the case of paleoclimate reconstruction, the δD of soil water is another unknown variable. Therefore, the claim that the isotopic composition of cellulose can be used as paleohygrometer (35) appears not to have strong support either from theory or from field data.

On the other hand, despite the fact that the hydrogen model does not contain a temperature-dependent fractionation term and the temperature-dependent fractionation of CO_2 involved in the oxygen model is small, temperature (both the growing season and the annual) has a relatively good correlation with isotopic compositions of cellulose. In particular, temperature shows excellent linear correlation with δD values of cellulose as well as δD values of soil water. In spite of the relative humidity effects on δD values of cellulose, δD values of cellulose still show excellent correlation with temperature. This suggests that not only δD of soil water but also the relative humidity has a functional relationship with temperature at the site.

A number of researchers have shown that diurnal changes in relative humidity are caused by variation in temperature at the site and that there exists a negative correlation between them (37,20). The average regression coefficient calculated from their data turns out to be approximately $-0.06^{\circ}/_{\circ\circ}/^{\circ}C$. Utilizing this value, an attempt has been made to examine this temperature effect via relative humidity on δD of cellulose in the model developed here. First of all, δD values of cellulose in trees from various localities are considered. Since the geographical relative humidity distribution is not a function of temperature, the overall δD values of cellulose from various sites become a function of temperature only. This is indeed confirmed by the empirical equation

$$\delta D_{cell} = 5.5\bar{t}_a - 130°/_{\circ\circ} \quad [88]$$

which has the same temperature coefficient (5.5°/₀₀/°C) as that of the meteoric water equation

$$\delta D_{mw} = \delta D_{sw} = 5.6\bar{t}_a - 100°/_{\circ\circ} \, . \quad [89]$$

Secondly, when δD values of cellulose from a single tree are considered, the negative temperature coefficient associated with relative humidity must be taken into consideration. Thus,

$$\delta D_{cell} = 5.6\bar{t}_a + 6.2\bar{t}_s + E. \quad [90]$$

In the case of the oxygen model, the equation reduces to

$$\delta^{18}O_{cell} = 0.43\bar{t}_a + 1.07\bar{t}_s + E. \quad [91]$$

The resultant temperature coefficients would vary from 5.6 to 12 for the hydrogen model and from 0.43 to 1.5 for the oxygen model depending upon the contribution from the growing season temperature terms. The dual temperature scales in equations [90,91] may introduce complications to interpretation of the isotope-temperature relationship. In general, t_a and t_s vary in the same direction reinforcing temperature coefficients. This phenomenon accounts for the increased temperature coefficients of both the hydrogen and oxygen isotopic compositions of the three spruce samples from Edmonton (see Tables 19 and 27). The measured temperature coefficient of hydrogen equations for WS1, WS2 and WS3 are 7.3, 13 and 15 respectively. The oxygen temperature coefficients for the equivalent samples are 1.36, 1.03 and 1.47 respectively.

It is apparent that δD values of cellulose in tree rings reflect mainly δD values of soil water. Superimposed upon that, temperature effects via relative humidity also play an important role in determining δD values of cellulose. The dual temperature scales involved in equations [90] and [91] may sometimes result in scattering of data if the relative humidity has no functional relationship with temperature. But the high temperature coefficients shown by individual trees from Edmonton indicate that temperature effect via relative humidity is generally in phase with temperature effect via hydrogen isotope fractionation of soil water and thereby increases the overall temperature coefficient.

In spite of the dual temperature scales, δD values of cellulose can be generally interpreted in terms of temperature. Therefore temperature is the best information one can derive from isotopic analysis of cellulose in plant materials. The significance of equations [90] and [91] is that, unlike relative humidity, temperature information can be obtained without any prior knowledge of the isotopic composition of the soil water. This is particularly important in the case of paleoclimatic reconstruction because the isotopic composition of the soil water for a given site is not known.

The results of this study suggest that cellulose in tree rings contain isotope thermometers (D and ^{18}O) whereby paleotemperature variation of the environment can be reconstructed. However, care should be taken in interpretation of the isotope data. The isotope data obtained from a tree may not be the representative of other trees. For example, WS1 and WS2 exhibited systematic difference in δD values of cellulose for the time equivalent rings despite the fact that both trees came from practically the same site. This suggests that there may exist biological fractionation in isotopic composition between individuals as well as species. When paleotemperature reconstruction is sought, it is desirable to analyze sequences of tree rings from a single tree. When isotope data from more than one tree are combined, it must be ascertained that the two trees are overlapped for some growth period and that the time equivalent tree rings have similar isotopic compositions or corrections must be made.

CHAPTER V

SUMMARY AND CONCLUSIONS

1. A new method of measuring both D/H ratios of $^{18}O/^{16}O$ ratios in organic compounds has been developed. This technique involves pyrolysis of organic compounds in a nickel reaction vessel. The pyrolysis method gives reproducible and accurate results with a variety of organic compounds and standard waters. The average precision of hydrogen isotopic analysis is ±2%. At present the method is limited to the organic compounds whose carbon-oxygen ratio is less than 1, due to formation of tar during early stage of pyrolysis. However, introduction of an oxidizing agent or free oxygen into the pyrolysis may remove this limitation.
2. Use of the nitration technique eliminates the exchangeable OH hydrogen without affecting the original isotopic composition of non-exchangeable CH hydrogen in cellulose. Pyrolysis of cellulose nitrate in the nickel reaction vessel shows no indication of tar formation indicating completion of reaction.
3. Nitration of cellulose extracted from wood by the sodium chlorite method is superior to the direct nitration of wood followed by acetone dissolution in terms of reproducibility and correlation with climatic *parameters. The direct nitration of wood*

produces cellulose nitrate whose δD value is systematically lower than that of nitrated cellulose extracted by sodium chlorite method.

4. δD values of cellulose nitrate in a long sequence of tree rings from single trees contain two different isotope scales. All three spruce samples WS1, WS2 and WS3 from Edmonton clearly show a sudden shift in δD values between the juvenile and mature sections of tree rings. In all cases, juvenile sections are systematically depleted in deuterium compared to mature sections. The reason for this is not completely understood. It may be attributed to unknown hormonal or biological metabolism associated with the tree growth, with results in changes in relative amount and structure of chemical constituents in tree rings. Such changes in turn may give rise to large variation of D value. When paleoclimate reconstruction is sought, the juvenile sections of tree ring sequence should be eliminated from the analysis. The differentiation of juvenile section from mature one is possible using ring width data.

5. The isotopic composition of cellulose in tree rings preserves a functional relationship with climatic parameters. δD values in cellulose nitrate from a long sequence of tree rings show a strong linear correlation with temperature at the growth site. On the other hand, $\delta^{18}O$ values of cellulose are found to

to be less well correlated with temperature. The choice of mean annual temperature is rather subjective. The mean annual temperature calculated from September of 2 years prior to the growth year to August of the previous year shows the best correlation with isotope data of tree rings. In other words, it is the mean annual temperature of the previous year which shows the best correlation with both δD and $\delta^{18}O$ values of tree rings.

6. For the three spruce samples analyzed, winter temperature plays a more important role in determining δD and $\delta^{18}O$ of cellulose than the temperature of the growing season. This conclusion together with the previous one indicates that the isotopic composition of soil water is the primary factor controlling the isotopic composition of tree rings and that soil water taken by the trees consists mainly of winter precipitation. This conclusion is further supported by the fact that the amount of precipitation shows a negative correlation with the isotope data. The negative correlation in turn is due to the amount effect of meteoric water on the isotopic compositions of precipitation. However, the preferential association of the isotopic composition with a particular seasonal temperature may be a growth site effect that is controlled by factors such as lithology and permeability of soil strate, and the topography of

the site.

7. Among the various chemical constituents in wood, cellulose shows the best correlation with temperature. δD values of extractive free wood show no significant correlation with temperature. δD values of lignin calculated from the isotope data of cellulose and extractive free wood also show poor correlation with temperature. Hydrogen isotope fractionation between lignin and cellulose shows poor correlation with temperature indicating the absence of a thermodynamic thermometer in the lignin-cellulose pair.

8. Ring width data show no significant correlation with isotope data or with mean annual temperature.

9. Hydrogen and oxygen isotope models have been developed to examine the role of individual climatic parameters in determining the isotopic composition of tree rings. The model equations describing the isotopic compositions of tree rings are given by

$$\delta D_{cell} = \delta D_{sw} + \varepsilon^* + \varepsilon_k + (\delta_A - \varepsilon_K)h + E \qquad [91]$$

$$\delta^{18}O_{cell} = 620 \ln\alpha_{CO_2-H_2O} + 0.62\{\delta^{18}O_{sw} + \varepsilon^* + \varepsilon_k + (\alpha_A - \varepsilon_k)h\} + E \qquad [92]$$

The models developed here are in excellent agreement with the isotope data. The working model equations which describe most of the natural isotope signature in trees is given by

$$\delta D_{cell} = \delta D_{sw} - 104h \pm 69 \qquad [93]$$

$$\delta^{18}O_{cell} = 620 \ln_{CO_2-H_2O} + 0.62(\delta^{18}O_{sw}+33.6-32h)+2.5 \qquad [94]$$

10. It is apparent that δD values of non-exchangeable hydrogen in cellulose of tree rings reflect mainly δD values of soil water. However, in addition to this growing season temperature via relative humidity also plays an important role. There is no indication of temperature-dependent hydrogen isotopic fractionation occurring during photosynthesis.

11. The oxygen in cellulose appears to originate from CO_2 which is in complete equilibrium with leaf water. Thus the oxygen model contains temperature-dependent fractionation factors. Due to the conflicting effects of temperature via CO_2-H_2O fractionation, soil water-vapor fractionation and relative humidity, oxygen shows a poorer correlation with temperature than does hydrogen.

12. The hydrogen isotope enrichment of cellulose with respect to soil water is a linear function of relative humidity at the site. Thus, relative humidity of a given site can be estimated by measuring the δD of tree rings and the δD of water taken by the tree. They are related by the equation

$$\Delta\delta D = \delta D_{cell} - \delta D_{sw} = -104h + 69 \qquad [95]$$

13. The relationship between δD and $\delta^{18}O$ values in cellulose varies depending upon the association of relative humidity with temperature. When relative humidity is linearly related to temperature at the site the relationship is given by

$$\delta D_{cell} = (5.2\pm 2)\delta^{18}O_{cell} + E. \qquad [96]$$

This is generally the case where isotope data are obtained from a single tree. When relative humidity has no such functional relationship with temperature this becomes

$$\delta D_{cell} = 13\delta^{18}O_{cell} - 432 \qquad [97]$$

The isotope data collected from a variety of growth sites belong to this category.

14. The best information one can obtain from isotope data of tree rings is mean annual temperature. However, interpretation may be complicated by the effect of relative humidity. The relationship between δD cell and temperature is given by

$$\delta D_{cell} = 4.6\ \bar{t}_a + 6.2\ \bar{t}_s + E \qquad [98]$$

where the term 6.2t is the contribution from relative humidity. In general, the changes in the growing season and mean annual temperature are in the same direction. Thus trees from single site will have a temperature coefficient bigger than 5.6. But mean δD values of tree rings from various sites will have a value close to 5.6.

15. The fact that the two trees from the same locality showed systematic difference in δD values of cellulose indicates that absolute temperature scale is not guaranteed from isotope data. Nevertheless, the temperature variation can be obtained from D/H ratios of non-exchangeable hydrogen in cellulose in a sequence of tree rings. When paleoclimate reconstruction is desired, hydrogen isotopic composition of cellulose nitrate in a long sequence of tree rings from a single tree should be analyzed. When isotope data from more than one tree are combined, the correction has to be made in such a way that the time equivalent ring section have the same isotopic composition.

3

RECOMMENDATION

The ultimate purpose of isotope studies in climatology is to obtain paleo-temperature variation over the last few thousand years. The information will form a basis which enables a better understanding of the natural mechanisms of climatic variation to be obtained and thereby future climate predictions made possible. The present study clearly shows that isotope records in tree rings can provide such information. Therefore, detailed isotopic records preserved in a long sequence of tree rings should be measured. Spectral analysis of isotope data thus obtained will show the cyclic nature of climatic variations. The sampling interval of tree rings, however, should be chosen in such a way that the desired periodicity can be resolved.

Present isotope technique provides only temperature variation. As for the absolute temperature scale, it is apparent that further work must be done. Absolute temperatures can be determined only from two co-existing substances formed in equilibrium with each other. Such systems may be found in cellulose. There are two types of hydrogen in cellulose. One is carbon-bound hydrogen and the other is oxygen-bound hydrogen. Carbon bound hydrogen is stable whereas oxygen bound hydrogen exchanges isotopically with water. However, about 25% of hydroxyl hydrogen which is in crystalline regions is stable. Thus if a significant fractionation exists between carbon bound hydrogen and non-exchangeable hydrogen in crystalline regions, an absolute temperature scale may be obtained by measuring the isotopic composition of CH hydrogen by the nitration method and OH hydrogen by the equilibration method.

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APPENDIX 1

Hydrogen Data (SMOW)

	Sample	year group	Temp°C	δD(SMOW)
1	WS1	1883-1888	1.86	-168
2		1888-1893	2.60	-155
3		1893-1989	2.34	-157
4		1998-1903	2.97	-151
5		1903-1908	3.20	-133
6		1908-1913	2.78	-133
7		1913-1918	2.70	-137
8		1918-1923	2.57	-134
9		1923-1928	2.59	-134
10		1928-1933	3.14	-131
11		1933-1938	2.14	-139
12		1938-1943	2.77	-132
13		1943-1948	2.89	-131
14		1948-1953	2.04	-141
15		1953-1958	3.05	-134
16		1958-1963	3.41	-129
17		1963-1968	2.81	-134
1	WS2	1888-1893	2.60	-146
2		1893-1898	2.34	-148
3		1898-1903	2.97	-139
4		1903-1908	3.20	-116
5		1908-1913	2.78	-116
6		1913-1918	2.70	-122
7		1918-1923	2.57	-121
8		1923-1928	2.59	-122
9		1928-1933	3.14	-116
10		1933-1938	2.14	-130
11		1938-1943	2.77	-119
12		1943-1948	2.89	-120
13		1948-1953	2.04	-130
14		1953-1958	3.05	-118
15		1958-1963	3.41	-111
1	WS3	1903-1908	3.20	-144
2		1908-1913	2.78	-159
3		1913-1918	2.70	-123
4		1918-1923	2.57	-130
5		1923-1928	2.59	-129
6		1928-1933	3.14	-118
7		1933-1938	2.14	-132
8		1938-1943	2.77	-118
9		1943-1948	2.89	-122
10		1948-1953	2.04	-132

11	1953-1958	3.05	-119
12	1958-1963	3.41	-112
13	1963-1968	2.81	-122
14	1968-1973	2.80	-121

APPENDIX 2

Oxygen Data (SMOW)

	Sample	year group	Temp°C	$\delta^{18}O$(SMOW)
1	WS1	1883-1888	1.86	23.86
2		1888-1893	2.60	24.13
3		1893-1898	2.34	24.20
4		1898-1903	2.97	23.77
5		1903-1908	3.20	24.44
6		1908-1913	2.78	24.62
7		1913-1918	2.70	24.47
8		1918-1923	2.57	24.52
9		1923-1928	2.59	24.05
10		1928-1933	3.14	25.72
11		1933-1938	2.14	23.68
12		1938-1943	2.77	23.57
13		1943-1948	2.89	23.45
14		1948-1953	2.04	22.82
15		1953-1958	3.05	24.91
16		1958-1963	3.41	24.89
17		1963-1968	2.81	24.46
1	WS2	1888-1893	2.60	24.57
2		1893-1898	2.34	24.27
3		1898-1903	2.97	25.73
4		1903-1908	3.20	25.58
5		1908-1913	2.78	25.4
6		1913-1918	2.70	25.25
7		1918-1923	2.57	24.57
8		1923-1928	2.59	25.02
9		1928-1933	3.14	25.90
10		1933-1938	2.14	24.98
11		1938-1943	2.77	24.76
12		1943-1948	2.89	25.13
13		1948-1953	2.04	23.72
14		1953-1958	3.05	25.21
15		1958-1963	3.41	25.36
1	WS3	1903-1908	3.20	23.73
2		1908-1913	2.78	24.58
3		1913-1918	2.70	24.98
4		1918-1923	2.57	24.22
5		1923-1928	2.59	24.44
6		1923-1928	2.59	25.45
7		1928-1933	3.14	23.25
8		1933-1938	2.14	23.97
9		1938-1943	2.77	24.9
10		1943-1948	2.89	23.78

11	1948-1953	2.04	25.46
12	1953-1958	3.05	25.21
13	1958-1963	3.41	24.74
14	1963-1968	2.81	23.52
15	1968-1973	2.80	24.45

APPENDIX 3

Direct Nitration of Wood (W-C-N)

WS1 (#0 #17)

	δD(SMOW)	Temp°C
1	-146	2.81
2	-139	3.41
3	-142	3.05
4	-150	2.04
5	-137	2.89
6	-137	2.77
7	-148	2.14
8	-139	3.14
9	-143	2.59
10	-142	2.57
11	-148	2.70
12	-141	2.78
13	-142	3.20
14	-157	2.97
15	-162	2.34
16	-16[illegible]	2.60
17	-171	1.86

$\Delta\delta D = \pm 2.4$ (Average)